NOV 0 9 1987

IVOMEC® (ivermectin)
1% Injection for Swine

Amendment to the
Environmental Assessment

1. Date: November 9, 1987

2. Name of applicant/petitioner: Merck & Co., Inc.

3. Address P.O. Box 2000
Rahway, NJ 07065

4. Description of the proposed action:

Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., supplements NADA 135-008, IVOMEC (ivermectin) 1% Injection for Swine with data demonstrating that the product used at its recommended dosage rate of 300 µg/kg is efficacious against somatic larvae of the threadworm Strongyloides ransomi.

Approval of this supplement is needed to inform the swine producer that the recommended IVOMEC treatment program controls somatic larvae of Strongyloides ransomi

6. Introduction of substances into the environment

It is anticipated that approval of this supplement will have no major effect on the introduction of substances into the environment either as a result of the manufacture of IVOMEC 1% Injection for Swine or as a result of its use and disposal.

The conditions conducive to Strongyloides ransomi infection have been described as follows:

"Strongyloides ransomi has a world-wide distribution but is less common in temperate areas. Infections are associated with poor hygiene, when pigs are kept in dirty pens and damp bedding, giving, in warm climates or in warm, unventilated houses, optimal conditions for development of the preparasitic phase and for exposure of the skin to infection"[1]

[1]Dunn, Angus M. "Veterinary Helminthology," 2nd ed., Published by William Heinemann Medical Books, LTD, London, England, 1978

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These conditions are conducive to infection by virtually all nematodes and it would be extremely unlikely to find swine infected solely with S. ransomi.

Since many of the swine bearing somatic larvae of S. ransomi infections are currently being treated with IVOMEC for control of other nematodes, approval of this supplement is unlikely to increase significantly the number of swine treated with IVOMEC. In addition, the present supplement does not alter the recommended frequency of treatment. Thus, approval of the present supplement is unlikely to have a major effect on the usage of IVOMEC for swine.

11. Alternatives to Proposed Action

Approval of the supplement is expected to have a minimal effect on the usage of IVOMEC 1% Injection for Swine. In the event this supplement is not approved, swine producers will not know that measures in addition to the recommended treatment program with IVOMEC are unnecessary for control of the somatic larvae of S. ransomi.

12. List of preparers

A. Foster

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13. Certification

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of the firm or agency responsible for preparation of the environmental assessment.

NOV 0 9 1987 (DATE)

Kenneth L. Davis (SIGNATURE)

# Environmental Assessment
# IVOMEC (ivermectin) Injection for Swine

A. June 30, 1985

B. Merck Sharp & Dohme Research Laboratories
Merck & Co., Inc.

C. P.O. Box 2000
Rahway, NJ 07065

D. Environmental Information

1. Describe the Proposed Action

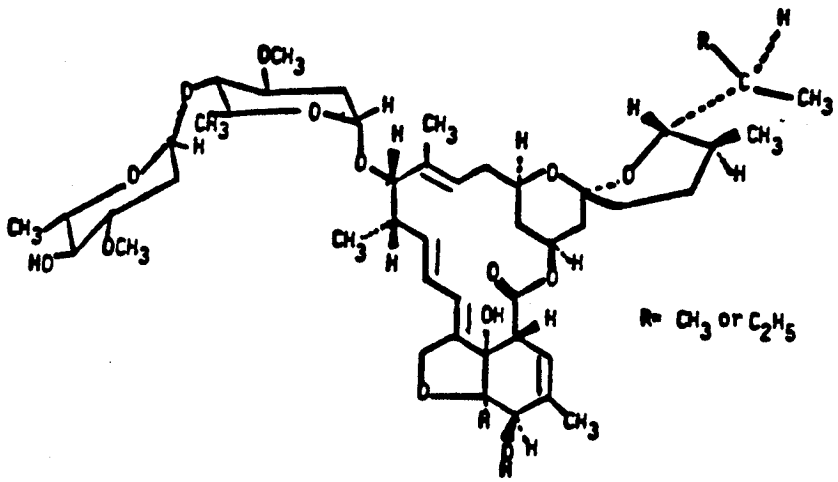
(a) Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., has filed a New Animal Drug Application for IVOMEC (ivermectin, MSD) Injection for Swine to be administered by subcutaneous injection at a dose rate of 300 mcg/kg of body weight for the treatment and control of the following internal and external parasites: Large Roundworm (Ascaris suum), adults and fourth stage larvae; Red Stomach Worm (Hyostrongylus rubidus), adults and fourth stage larvae; Nodular Worm (Oesophagostomum spp.), adults and fourth stage larvae; Intestinal Threadworm (Strongyloides ransomi), adults; Lungworm (Metastrongylus spp.), adults; Swine Louse (Haematopinus suis); Mange Mite (Sarcoptes scabiei var. suis).

A drug withdrawal period of 18 days prior to slaughter of swine for food has been established.

1. Describe the Proposed Action

Treatment may be repeated at intervals of not less than 18 days; however, rate of parasite reinfestation, prudent husbandry practices and economic considerations would dictate that most market swine would be treated once during the life of the animal. Swine kept for breeding purposes might be treated once or twice yearly throughout their lives.

(b) Physical and Chemical Properties are as Follows:




| Empirical Formula | Molecular Weight |
|---|---|
| (R = $C_2H_5$) $C_{48}H_{74}O_{14}$ | 875.10 |
| (R = $CH_3$) $C_{47}H_{72}O_{14}$ | 861.07 |

1. Describe the Proposed Action

(b) Physical and Chemical Properties (Cont'd)

Ivermectin is produced by fermentation and subsequent chemical hydrogenation and is a mixture of two closely related homologues belonging to a class of compounds known as avermectins. The chemical names of the two homologues are: 22,23-dihydro-avermectin $B_{1a}$ (R = $C_2H_5$) and 25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl) avermectin $B_{1a}$ (R = $CH_3$). The latter is also known as 22,23-dihydro-avermectin $B_{1b}$.

Ivermectin contains at least 80% of the compound in which R in the above structure is the ethyl group and less than 20% of the compound in which R is the methyl group. It is a white to yellowish white crystalline powder and has an ill-defined melting point of about 150°C. The material is optically active and has a specific rotation $[\alpha]\ ^{25°C}_{D}$, ~-19° (C=0.5, $CH_3OH$). The ultraviolet absorption spectrum in methanol is characterized by maxima at 237, 245 and 253 nm with A1%1cm values of about 349, 382 and 248, respectively.

1. Describe the Proposed Action

(b) Physical and Chemical Properties (Cont'd)

The photodegradation studies of $^{14}C$-avermectin $B_{1a}$ in aqueous suspension were carried out under sunlight. The half-lives for avermectin $B_{1a}$ in duplicate tests were found to be 3.5 and 12 hours. The photodegradation studies of $^{3}H$-avermectin $B_{1a}$ in soil thin layer plates were carried out under sunlight. The half-life for avermectin $B_{1a}$ was found to be 21 hours. Ivermectin is very insoluble in water, the concentration of a saturated aqueous solution being 5 ppm. Ivermectin is freely soluble in methanol, chloroform, p-dioxane, dimethylformamide and ethyl acetate; soluble in 95% ethanol, diethyl ether, methylene chloride, acetone, and aromatic hydrocarbons; and very slightly soluble in aliphatic hydrocarbons. The infrared and nuclear magnetic absorption spectra are consistent with proposed structures.

Ivermectin has been shown to be stable for at least six months when stored under ambient conditions.

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1. Describe the Proposed Action

(b) Physical and Chemical Properties (Cont'd)

Ivermectin contains at least 95% of the two compounds shown above as determined by UV absorption and liquid chromatography.

Based on radioactivity measurements, the octanol coefficient for ivermectin is 1651; i.e.,

$$K_D \text{ of } \frac{\text{octanol}}{\text{pH 7 buffer (or water)}} = 1651$$

This indicates a strong affinity of ivermectin for lipid systems, but the residue data contained in the New Animal Drug Application show a rapid depletion of drug and metabolites from animal fat.

The soprtion and desorption of tritium-labeled ivermectin with an Iowa soil of clay loam texture was investigated. Samples containing 1.5 g soil, 7.5 ml 0.01 M $CaCl_2$ solution, and ivermectin were mixed for 16 hours, then centrifuged. The ivermectin in solution was determined by counting an aliquot, while the

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1. Describe the Proposed Action

(b) Physical and Chemical Properties (Cont'd)

ivermectin bound was determined by combustion of the soils. The soil samples were desorbed twice by replacing the equilibrated solution with fresh $CaCl_2$ solution. The Freundlich binding parameters were determined for the adsorption and desorption steps. Since the adsorption and desorption values were similar, overall Freundlich binding values of K = 207 and n = 1.16 were determined. The distribution coefficient ($K_D$) for adsorption and two desorptions were respectively 282.6, 348.9, and 366.5. The overall $K_D$, averaging adsorption and desorption data was 332.7. From this value, the constant for binding to organic carbon ($K_{oc}$) can be calculated as $K_{oc}$ = 100 x $K_D$/% organic carbon or $K_{oc}$ = 100 x 332.7/2.645 = 12578, and where % organic carbon = % organic matter/1.724.

(c) Pharmacology

Ivermectin inactivates nematodes, arachnids and insects. Its action on the nematodes is by inhibiting signal transmission from the ventral cord interneurons

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

to the excitatory motor neurons. It acts by stimulating the release of the inhibitory neurotransmitter gamma-aminobutryic acid (GABA) from presynaptic nerve terminals as well as by potentiating GABA binding to the post synaptic receptors. The ivermectin-treated nematodes lose central command to move. Ivermectin acts on the arthropods by inhibiting signal transmission at the neuromuscular junctions via the same mechanism of amplifying GABA action. The treated arthropods become paralyzed.

Ivermectin and the avermectins are not effective against flukes and tapeworms, in which GABA is not found as a neurotransmitter.

In a laboratory screen, a mixture of at least 80% avermectin $B_{1a}$ and not more than 20% avermectin $B_{1b}$, was given by gavage to rodents harboring 3-week-old Fasciola hepatica infections. Five control rodents had 1 to 3 worms at necropsy four days after

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

treatment, while two animals dosed at 2.5 mg/kg of the avermectins had 2 and 3 worms each. In a similar screen using the tapeworm Hymenolepis diminuta, laboratory rodents harboring 14-day-old worms were given placebo, commercial yomesan at 37.5 mg/kg as a positive control or ivermectin at 1 mg/kg. Necropsy 6 days after treatment indicated 3 to 5 worms in each of the four animals receiving the placebo, zero worms in the three animals receiving yomesan, and 2, 4 and 6 worms in the three animals receiving ivermectin.

Also in field trials, ivermectin at 50 to 400 mcg/kg had no effect against the tapeworms Dipylidium caninum and Taenia spp. in dogs.(1)* Similarly, in anthelmintic tests in equids, ivermectin was shown to be ineffective against natural tapeworm infections.(2)

*Literature references on page 159.

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1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

Ivermectin is unrelated structurally to any of the present available parasiticides. Because of this and its unique mode of action not shared by any other parasiticides, cross-resistance is not expected to occur.

Ivermectin has mild anticonvulsant activity in the pharmacometric screen of central nervous system effects in the mouse. The $LD_{50}$ 24 hours after drug administration was estimated to be less than 10 mg/kg. Ivermectin was virtually inactive against electroshock and bicuculline-induced convulsions one hour following treatment. However, anticonvulsant activity of ivermectin increased markedly in both assays when measured 4 hours after treatment.

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

At a dose of 0.5 mg/kg I.V., ivermectin had no significant effect on blood pressure or heart rate of anesthetized dogs, nor did it modify blood pressure or heart rate responses to autonomic drugs in a standard assay. The $H_2B_{1a}$ component of ivermectin enhanced the $^3H$-diazepam binding in rat brain $P_2$ membranes by 32% at a concentration of 1 mcM.

Ivermectin, at an intragastric dose of 0.5 mg/kg, did not affect evoked or basal gastric secretion in dogs with a chronic gastric fistula.

Ivermectin, at 1 and 2 mg/ml (parts per thousand), did not inhibit the growth of 9 bacterial or 5 fungal strains. The bacterial strains were Staphylococcus aureus, Streptococcus pyogenes, Bordetella bronchseptica, Klebsiella pneumoniae, Aerobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

(two cultures), Proteus mirabilis. The fungal strains were Alternaria, Fusarium, Cephalosporium, Pullularia pullulans, and Aspergillus niger. The solvent for ivermectin, DMSO, was present at a level of 1%. This level of DMSO also had no effect on the growth of the bacterial and fungal cultures. Ivermectin was also tested in the antibacterial agent screen at 1 and 2 mg/ml against 5 strains each of Escherichia coli and Salmonella typhimurium of animal origin (calf and pig animal sources). The solvent, DMSO, was again present at a level of 1%. Neither the ivermectin nor the DMSO had any inhibitory effect towards growth of any of the test organisms at these levels.

Ivermectin displays no substantial activity against protozoa. In an in vitro assay using Trichomonas foetus, the major isomer of ivermectin, $H_2B_{1a}$, displayed some activity in reducing T. foetus growth,

1. Describe the Proposed Action

(c) Pharmacology (Cont'd)

but only at 100 mcg/ml in a stock solution. Concentration of $H_2B_{1a}$ at 0.2 to 50 mcg/ml were not effective in the 40-hour assay, whereas the 100 mcg/ml level of $H_2B_{1a}$ was effective in only two out of three assays. Avermectin $B_{1a}$ was inactive in 1, 10 and 100 mcg/ml. Similarly, in an in vitro assay using Trypanosoma brucei, $H_2B_{1a}$ again displayed growth inhibition in a 6-hour incubation at 100 mcg/ml, but no activity at 1 or 10 mcg/ml. Here again, avermectin $B_{1a}$ was inactive at 1, 10 and 100 mcg/ml. In an in vivo assay of T. brucei in mice, doses of 50 mg/kg of $H_2B_{1a}$ and avermectin $B_{1a}$ were toxic. Doses from 0.4 to 10 mg/kg produced some toxic reactions. Over the dosing range of 0.1 to 10 mg/kg, $H_2B_{1a}$ and avermectin $B_{1a}$ provided no in vivo protection against T. brucei infection.

1. Describe the Proposed Action

(d) Toxicity

Ivermectin has been shown to be negative in the Ames Microbiological Mutation Assay and in a Mammalian Mutation Assay using a mouse lymphoma cell line. In addition, ivermectin did not induce unscheduled DNA synthesis in a human fibroblast cell culture. The results of these studies showed no genotoxic hazard associated with the use of ivermectin.

Ivermectin is teratogenic in rats, rabbits and mice at or near maternotoxic dose levels. Evidence of a teratogenic effect was limited to cleft palate that occurred at a low frequency in all three species and clubbing of the forepaws which occurred only in the rabbit fetuses. Mice are the species most sensitive to the effects of ivermectin with maternotoxicity at a dose of 0.2 mg/kg/day and teratogenicity at 0.4 mg/kg/day. A dose of 0.1 mg/kg/day was without maternotoxic or teratogenic effect in mice. In rabbits, 6 mg/kg/day was maternotoxic and teratogenic,

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

and teratogenicity was also evident at a dose of 3 mg/kg/day. A dose of 1.5 mg/kg/day in the rabbit was without maternotoxic or teratogenic effect. The threshold for maternotoxicity and teratogenicity in rats was 10 mg/kg/day; a dose of 5 mg/kg/day was neither maternotoxic nor teratogenic.

In a reproduction study in rats, as well as in acute studies, it was demonstrated that neonates are significantly more susceptible to the toxic effects of ivermectin than adult animals. The $LD_{50}$ for infant rats is approximately 10-fold less than that of adults. In a rat reproduction study, there was increased neonatal mortality at a dose of 1.6 mg/kg/day. In a 14-week oral toxicity study in which weanling rats (about 4 weeks of age) derived from the reproduction study were given ivermectin at doses up to 1.6 mg/kg/day. There was no treatment-related mortality.

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

In the 14-week oral toxicity study in rats mentioned above, no treatment-related effects were observed at a dose of 0.4 mg/kg/day. At doses of 0.8 and 1.6 mg/kg/day, enlarged spleens resulting from congestion and extramedullary hematopoiesis occurred in a few rats. This was accompanied by the accumulation of iron-positive pigment in the renal tubular epithelium and hyperplasia of the bone marrow.

In a 14-week oral toxicity study in dogs, no treatment-related effects were observed in animals given 0.5 mg/kg/day. Dogs given 1 and 2 mg/kg/day developed mydriasis and lost a small amount of weight. Four of 8 dogs given 2 mg/kg/day developed tremors, ataxia and anorexia and became dehydrated. These dogs were killed prior to termination of the study, and agonal gastrointestinal hemorrhage and/or congestion was observed in 2 of the dogs. No other treatment-related histologic change was observed in any dogs.

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

The clinical signs of acute toxicity caused by ivermectin in the pig are lethargy, followed by ataxia, mydriasis, intermittent tremors, labored breathing and lateral recumbency. These signs appeared in pigs injected once subcutaneously with ivermectin at 30 mg/kg body weight (100 times the recommended use level). Pigs treated once with ivermectin at levels up to 15 mg/kg body weight (50 times the recommended use level) did not exhibit signs of toxicity. Observations were made for 3 weeks following treatment.

Thirty-two cattle were used in 2 trials of 21 days duration to examine the effects of ivermectin doses up to 8,000 mcg/kg as a single subcutaneous injection on Day 1. Deaths occurred in 3 of 4 animals at this dose rate, but no clinical signs of toxicosis were seen at 6,000 mcg/kg, which is 30 times use-level dose rate.

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

Trials indicate that signs of toxicity (partial mydriasis) may be seen in some horses at levels approximately 15 times the proposed use level as a single intramuscular injection, and toxicosis and some fatalities occurred in horses receiving single doses (12 mg/kg) in the vicinity of 60 times use level. Observations were continued for 21 days following injection.

Sheep given ivermectin orally in a micelle formulation in a 21-day trial did not evidence signs of serious reaction until the single dose (4 mg/kg) exceeded 20 times the use level.

The oral acute $LD_{50}$ of avermectin $B_{1a}$ in the mallard duck is 85 mg/kg, with 95% confidence limits, 67 to 120 mg/kg. At the lowest dosage level tested of 10.0 mg/kg, slight lethargy and loss of coordination occurred immediately after dosing and lasted through

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

day one. Toxicity symptoms were pronounced and lasted longer as the dosage levels of the drug increased. Symptoms of toxicity observed included lethargy, loss of coordination, prostrate posture, lower limb rigidity, loss of righting reflex and depression.

The subacute $LC_{50}$ in this avian species in an eight-day dietary study is 383 ppm, with 95% confidence limits, 302 ppm to 487 ppm.

At 162 ppm, the lowest concentration tested, lethargy, reduced reaction to external stimuli (sound and movement) wing droop, loss of coordination and lower limb weakness occurred within three hours of exposure to the treated diet, and lasted for five days. However, birds appeared normal 24 hours after being on basal diet; there was 20% mortality at the 288 ppm concentration level, 80% at the 511 ppm concentration level, and 100% at higher concentration levels.

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1. Describe the Proposed Action

(d) Toxicity (Cont'd)

Duration of toxicity symptoms increased as the levels of avermectin $B_{1a}$ increased.

The acute oral $LD_{50}$ of avermectin $B_{1a}$ in the bobwhite quail is estimated to be greater than 2000 mg/kg.

At an avermectin $B_1$ level of 62.5 mg/kg, the birds exhibited lethargy, reduced reaction to external stimuli (sound and movement) and loose droppings that lasted for two days. There also was no mortality at the lowest avermectin $B_1$ level (62.5 mg/kg) tested. At higher dose levels of avermectin $B_1$ (125 mg/kg), toxicity symptoms appeared within 1-2 hours, well established within the first day, and lasted for five days. These symptoms included loss of coordination, shallow and rapid respiration, depression, reduced reaction to external stimuli (sound and movement) wing droop, lower limb weakness, prostrate posture, ruffled

1. Describe the Proposed Action

(d) Toxicity (Cont'd)

appearance, lethargy, comatose state and some loose droppings. However, no mortalities occurred at the 125 mg/kg dose level. Mortality incidents were inconsistent with increased avermectin $B_1$ levels.

Avermectin $B_1$ dosage levels of 250 mg/kg and 1000 mg/kg caused only 10% mortality, but levels of 500 mg/kg and 2000 mg/kg caused 40% mortality. However, severity of the toxicity symptoms increased as Avermectin $B_1$ dosage levels increased.

In an eight-day dietary study in this species, the subacute $LC_{50}$ value of avermectin $B_{1a}$ was determined to be 3102 ppm, with confidence limits (95%) of 2338 to 4393 ppm.

The lowest concentration tested (288 ppm) induced toxicity symptoms within four hours of exposure to the treated diet. These toxicity symptoms included

1. Describe the Proposed Action

lethargy and reduced reaction to external stimuli (sound and movement) and lasted for three days after which birds appeared normal. Duration of the toxicity symptoms increased as a function of increased avermectin $B_1$ concentration in the diet. Mortalities occurred at concentration levels $\geq$ 1620 ppm.

(See table on following page -- 22.)

Table 1.
Results of Acute, Subacute, Oral Teratology, and Genotoxic Studies of Ivermectin

| TYPE OF STUDY | SPECIES | DURATION | SIGNIFICANT FINDINGS ANTEMORTEM | SIGNIFICANT FINDINGS POSTMORTEM | NO EFFECT LEVEL (mg/kg/day) |
|---|---|---|---|---|---|
| Acute Oral | Mouse (M,F) | - | - | $LD_{50}$ 11.6-41.6 mg/kg | - |
| Acute Oral | Rat (M,F) | - | - | $LD_{50}$ 42-53 mg/kg | - |
| Ocular Irritation | Rabbit | - | - | Slightly irritating | - |
| Dermal Irritation | Rabbit | - | - | Non-irritating | - |
| Dermal $LD_{50}$ | Rabbit | - | - | $LD_{50}$ 406 mg/kg | - |
| Dermal $LD_{50}$ | Rat | - | - | $LD_{50}$ 660 mg/kg | - |
| Acute Inhalation | Rat | 1 hr. exposure | Transient irritation mucous membranes | No deaths | Actual exposure based on respirable particles 0.4 mg/kg |
| Acute Subcutaneous | Young Dogs | - | - | $LD_{50}$ ±10 mg/kg | $LD_{50}$ 4.8 mg/kg |
| Toxicity | Rat | In utero phases | Hypothermia, absence of milk in epigastric region | Increased pup mortality | 0.8 |
| | | 14-Week phase | None | Enlarged spleen, possible intravascular hemolysis | 0.4 |
| Toxicity | Dog | 14-Week | Tumors, ataxia, dehydration, mydriasis | Dogs sacrificed due to poor condition, agonal changes in 2 dogs - no other changes | 0.5 |
| Teratology | Mouse | Day 6-15 of gestation | Mortality, tremors, convulsions, coma | Cleft palate | Maternal effects - 0.1<br>Teratogenic effect - 0.2 |
| Teratology | Rat | Day 6-17 of gestation | Sedation; 3 rats sacrificed in poor physical condition | Cleft palate | Maternal effects - 5.0<br>Teratogenic effect - 5.0 |
| Teratology | Rabbit | Day 6-18 of gestation | Sedation, decreased body weight | Decreased fetal weight, increased number fetal deaths, cleft palate, clubbed forepaws | Maternal effects - 3.0<br>Teratogenic effect - 1.5 |
| Teratology | Dog | Day 5, 15, 25, 35 or Day 10, 20, 30, 40 of gestation | None | No treatment- related external, visceral or skeletal malformations | 0.5 |
| Ames Bacterial Mutagen Assay | - | - | Negative | - | 2 mg/plate |
| Mouse Lymphoma Mutagen Assay | - | - | Negative | - | 80 g/ml |
| Unscheduled DNA Synthesis in Human Lung Fibroblast | - | - | Negative | - | 10 mg/flask |

1. Describe the Proposed Action

(e) Purpose and Benefits

The cost of parasitism, in terms of morbidity and resultant depression of growth and feed efficiency, has long been recognized as a significant factor in the economical production of pork products. Thus, the increased significance of parasitism has led to the widespread use of antiparasitic drugs. Losses to the swine industry have been primarily attributed to the loss in feed efficiency, due to internal parasites and the interruption of feeding habits caused by external parasite infestation.

The economic losses from the common swine ascarid (Ascaris suum) were estimated to be approximately $385 million in 1979.[17] Losses from other nematodes are influenced by geographical prevalence, management and nutrition but are believed to decrease profits annually by several millions of dollars. Economic losses from swine ectoparasites have not been adequately determined but significant increases in weight gain and feed

1. Describe the Proposed Action

(e) Purpose and Benefits (Cont'd)

efficiency have been observed following complete control of swine mange mites Sarcoptes scabiei var suis.[18] A broad spectrum antiparasitic drug that will control the major endo- and ectoparasites of swine coupled with sound management and nutrition will help reverse the economic losses that are due to parasitism.

Ivermectin is an effective, new antiparasitic agent which is not chemically related nor paralleled in its spectrum of activity to any other drug now being marketed. In the proposed form, ivermectin provides the most convenient, ready-to-use method of control without leaving hazardous or potentially dangerous wastes which require careful handling, storage, transport and disposal. Since IVOMEC is an injectable product, the environmental concern of disposing of "spent" dips and sprays is obviated.

1. Describe the Proposed Action

(e) Purpose and Benefits (Cont'd)

The unique activity of this product also permits control of external parasites of significance at times of the year when currently available products, such as dips and sprays, cannot be used. Clearly beneficial effects with economic value would result from its use, such as decreased morbidity, resultant increase in feed efficiency and environmental protection.

(f) Potential Market Handling and Storage

The marketplace for IVOMEC injection is presently segmented into two distinct entities; namely, the endoparasite or anthelmintic market and the ectoparasite market. Presently, the parasiticide market is being served by a multitude of products designed for either endo- or ectoparasite control. The ectoparasite market is characterized by many products and compounds of which there is no recognized market leader. IVOMEC injectable is expected to attain a significant market share within each market segment, due to its control of both ecto- and endoparasites.

1. Describe the Proposed Action

(f) Potential Market Handling and Storage (Cont'd)

Due to the nature of the ectoparasiticide market, an evaluation of the potential kilogram market for IVOMEC in swine is based solely on the endoparasiticide market. Based on current market research information, the kilogram potential for IVOMEC penetration into the swine market is estimated at about 10% of the combined horse and cattle usage annually.

There are no special handling or storage requirements for IVOMEC Injection for Swine. Stability studies show that IVOMEC Injection will be stable for two years when stored at room temperature.

The proposed trade channels for distribution of IVOMEC Injection will be similar to both anthelmintic and ectoparasiticide products. Animal health wholesalers, dealers, their distributors and licensed veterinarians will be utilized.

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc.

There are several reports in the literature describing the insecticidal as opposed to the parasiticidal activity of ivermectin and structurally related analogues, the avermectins.

The first report(3) tested the insecticidal activity of several avermectins against Tribolium confusum (confused flour beetle). Four avermectin analogues were 100% lethal by 28 days to T. confusum at 100 ppm, as compared to a 34% mortality in the controls. Malathion, the positive control, was more potent, showing similar activity at 10 ppm and less. This report and a second report(4) also reported on the ectoparasitic activity of avermectins on Cuterebra spp. (robust bot fly) larvae and Lucilia cuprina (sheep blow fly) larvae.

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc. (Cont'd)

A report by Putter, et al.[5] summarized the activity of avermectin $B_{1a}$ against several mites, pesticidal worms, beetles, aphids, ants, larval flies and mosquitoes, and nematodes. Avermectin was active against all motile mite stages, but had no ovicidal activity. The lethal action of avermectin was slower than that of conventional organophosphates and pyrethroid insecticides. In the fire ant, avermectin permanently halted egg production in the queen at 0.12 g/ha, but was not 100% lethal to the worker ants. Larval flies and mosquitoes exposed to 2 to 50 ppb in their rearing medium failed to pupate. Another avermectin, $B_{2a}$, controlled soil nematodes at a rate of 0.16 to 0.24 kg/ha and was not observed to be phytotoxic to greenhouse tomatoes and cucumbers at doses as high as 10 kg/ha. It was postulated that the avermectins inhibit nervous signal transmissions at the

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc. (Cont'd)

neuromuscular junctions of arthropods and block signal transmission from ventral interneurons to excitatory motor neurons in the nematodes.

Reports[6-8] on the larvicidal activity of ivermectin, MK-933, towards horn, stable, face and house flies have been published. The first of these determined the $LC_{50}$ and $LC_{90}$ concentrations of insecticides towards stable and horn flies in a larval medium of a dry mix, bovine feces and water. The larval susceptibilities were determined on the basis of emerging adults, corrected against the number of adults emerging from medium treated with acetone (insecticidal solvent) only. MK-933 displayed $LC_{90}$ values of 0.186 ppm for stable flies and 0.006 ppm for horn flies by this method. The second report looked at larvicidal activity of MK-933 in the feces of steers given daily oral or subcutaneous doses, or a single subcutaneous dose, or via a bolus. Daily oral doses as low as 20

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc. (Cont'd)

mcg/kg were sufficient to prevent development of the immature stage of the stable fly, while as little as 0.5 mcg/kg/day provided horn fly control. A single injection of 200 mcg/kg, the anthelmintic dosage, controlled horn flies in the manure for up to 4 weeks post treatment. Oral doses of 1 mcg/kg/day killed all horn fly larvae in the manure, while a 5 mcg/kg/day oral dose killed all the face flies, about 60% of the stable flies and 90% of the house flies in the manure. The third report on larvicidal activity of MK-933 reported that a 200 mcg/kg injection resulted in 100% corrected mortality of the face fly larvae developing in the feces for 9 days. Larvae emerging from feces sampled 10 to 15 days post treatment developed into malformed pupae, with approximately 90% failing to undergo eclosion. Effectiveness of ivermectin decreased after 15 days post treatment.

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1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc. (Cont'd)

Results of tests of avermectin $B_{1a}$ against the red imported fire ants, Solenopsis invicta Buren, have been reported.[9,10] The avermectin $B_{1a}$, fed to laboratory colonies at concentrations as low as 0.0025% in soybean oil bait, inhibited reproduction of queens. Some worker mortalities occurred at concentrations of 0.025% or greater. Field tests indicated only 8 out of 928 colonies that fed on bait applied at rates of 0.0077 to 7.41 g/ha had worker brood. The primary effect of avermectin $B_{1a}$ was on the reproductive capacity of the queen rather than acute toxicity for the workers. The damage to the queen was characterized by irreversible cell and tissue damage to the ovaries, resulting in complete sterility or reduction in the numbers and size of eggs laid.

The efficacy of avermectins for rootknot control in tobacco was reported.[11] Control of Meloidogyne incognita was studied in tobacco fields for two

1. Describe the Proposed Action

(g) Literature - Avermectins and Ivermectins as Insecticides, etc. (Cont'd)

seasons. Applications of 0.05 to 0.50 kg/ha suppressed root galling and egg production.

And finally, a recent article in Science summarized data on the microbiology, isolation and structure determination, chemistry, antiparasitic efficacy, mode of action, safety and metabolic disposition of the avermectin family of compounds.(12)

(h) Brief Description of Primary (and Secondary) Environment Affected

In the analysis of the potential adverse impact on the environment from treating swine with ivermectin, the following areas were examined and are reported in greater detail in Section D-2:

(1) The Environmental Burden

The expected environmental burden based on the concentration of ivermectin and its metabolites in

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

the accumulated waste in a swine feedlot both under normal practices and in a "worst-case" situation. This environmental burden is calculated as the soil concentration (ppb) when feedlot waste is spread on a field and mixed with the top 6 inches of soil.

(2) Stability in Soil

Half-life of ivermectin and ivermectin in steer feces was measured when treated soil was exposed to outdoor conditions in New Jersey, both in the winter and in the summer. Analysis of water percolated through these samples permitted characterization of the effluent.

(3) Stability in Water

Swine feces/water homogenates were prepared in a similar ratio as expected under feedlot

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

conditions. These homogenates contained drug-equivalent and ivermectin levels of 49 and 19 ppb, respectively; levels in excess of those calculated to be naturally occurring in swine feedlots.

Samples were stored in capped bottles, wrapped in foil to exclude light, and analyzed periodically. Analysis of the homogenates showed no measurable loss of ivermectin during a period of 42 days stored at room temperature.

(4) Soil Column Leaching

Experiments were carried out to determine the aqueous leaching of ivermectin and its metabolites from feces of swine dosed with ivermectin. Feces alone and feces mixed 1:1 (w/w) with Iowa silt loam soil (drug-equivalent 278 ppb) and placed on top of 2 cm. depth of this soil were leached for a

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1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

few days with water (1150 ml). The leachates were assayed for total percolated drug and metabolites, for percent of ivermectin in the leachates, and for toxicity of leachates towards Daphnia magna.

(5) Soil Sorption/Desorption

The sorption and desorption of tritium-labeled ivermectin with an Iowa soil of clay loam texture was investigated. Samples containing 1.5 g soil, 7.5 ml 0.01 M $CaCl_2$ solution, and ivermectin were mixed for 16 hours, then centrifuged. The ivermectin bound was determined by combustion of the soils. The soil samples were desorbed twice by replacing the equilibrated solution with fresh $CaCl_2$ solution. The distribution coeffieient ($K_D$) between bound and soluble ivermectin was determined. From this value, the constant for binding to organic carbon ($K_{oc}$) was calculated. The Freundlich binding parameters were also determined for the adsorption and desorption steps.

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

(6) Soil Toxicity -- Microbial Effects

Feces from steers dosed with ivermectin were mixed with either pasture or forest soil and the effects on soil nitrification and soil respiration were measured.

(7) Phytotoxicity of Ivermectin

A fresh-water, unicellular, non-motile chlorophyte, Chlorella pyrenoidosa was used in an algal toxicity test to measure the effect of ivermectin on overall cell growth, mean specific growth rate, maximum standing crop, algal biomass and lag period. Observations were also recorded relative to the phytotoxicity of avermectin, on a variety of food crops during the conduct of insecticidal efficacy studies.

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

(8) Toxicity to Aquatic Organisms

The toxicity of ivermectin toward three aquatic species was measured: the bluegill sunfish, rainbow trout and the crustacean, Daphnia magna or water flea.

(9) Toxicity to Nematodes, Arachnids and Insects

The effect of ivermectin and related compounds on a number of insects, phytophagous mites, and soil nematodes, was measured in a variety of tests.

(10) Toxicity to Annelids

Studies were conducted to determine the $LC_{50}$ for ivermectin to the manure worms, Eisenia foetida in artificial soil under controlled laboratory conditions.

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

From the results of these studies, it was concluded that the greatest potential for adverse environmental impact would be on aquatic organisms should ivermectin be permitted direct entry into ponds, streams, or rivers. The following statement was added to the labelling to avert such an action:

"ENVIRONMENTAL SAFETY

Studies indicate that when ivermectin comes in contact with the soil, it readily and tightly binds to the soil and becomes inactive over time. Free ivermectin may adversely affect fish and certain water-borne organisms on which they feed. Do not permit water runoff from feedlots to enter lakes, streams, or ponds. Do not contaminate water by direct application or by the improper disposal of drug containers. Dispose of empty containers in approved landfills or by incineration."

1. Describe the Proposed Action

(h) Brief Description of Primary (and Secondary) Environment Affected (Cont'd)

A secondary and minor potential adverse impact on the environment could occur in the manufacture of ivermectin and in formulating IVOMEC Injection for Swine. The environmental controls imposed during each of these operations at four locations have been examined and have been found to meet or exceed all of the requirements set forth by the respective governmental regulatory authorities.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden

The projected use of ivermectin in swine involves the subcutaneous administration of the drug at a level of about 0.3 mg/kg body weight. The animals may be contained in a pasture, a large commercial feedlot, a slotted-floor house or a solid concrete-floored facility. The dirt lot or pasture and the solid concrete-floored facility comprise the majority of the swine operations, and represent the extremes in dilution of the solid wastes for use as fertilizer. Thus, the environmental burden for these two cases will be discussed. In all cases, market hogs would generally receive 1 dose of ivermectin while breeder hogs would receive 2 during a 24-week period.

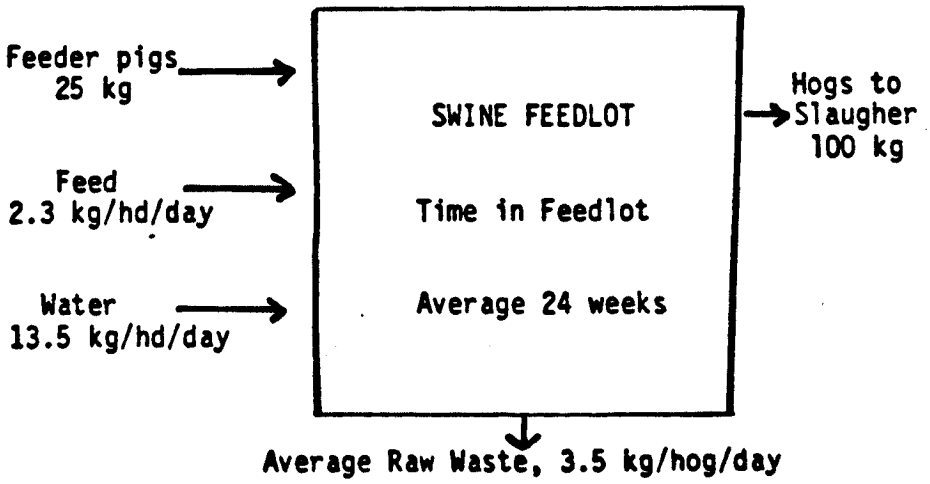
Figure 1 shows a flow diagram for a typical swine feedlot. A feeder pig would come into the feedlot at about 25 kg, would receive 1 dose of ivermectin and would spend an average of 24 weeks (168 days) in the feedlot before reaching market weight of approximately 100 kg.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

During that time, an average of 3.5 kg/hog/day of raw waste would be produced.[13] About 15% of the hogs are breeder hogs, which would be expected to receive about 2 doses of ivermectin in the 24-week period, and would average 150 kg body weight or more. A 150 kg hog would be expected to produce about 11.6 kg waste per day (Appendix 1). The manure from such a feedlot would be used for fertilizer at a level of about 5 to 60 tons/acre (Appendix 4 and References 16a through e).

Figure 1. TYPICAL SWINE FEEDLOT FLOW DIAGRAM

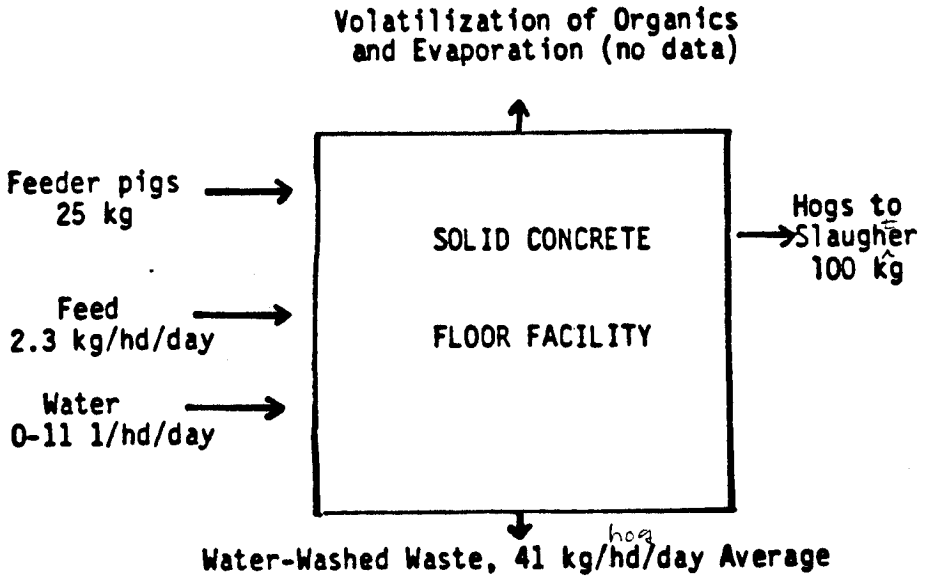


2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

Figure 2 shows a flow diagram for a typical solid concrete-floored facility. There, water-washed waste, comprised of feces and urine, would be hosed into pits or tanks and stored under unaerated conditions until it would be pumped out and spread onto fields at 3000 to 8000 gallons (11,000 - 30,000 liters) per acre and knifed into the ground (Appendixes 2 and 3).

Figure 2. Topical Solid Concrete-Floor Facility



2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

To determine the levels of ivermectin and its metabolites in the feces of dosed swine, twelve barrows were dosed subcutaneously with the drug at a level of 0.4 mg/kg body weight. The barrows ranged from 20 to 27 kg in body weight. Three barrows were slaughtered at each of four withdrawal periods: 1, 7, 14, or 28 days after dosing. Excreta were collected from three swine for seven days. The barrows excreted an average of 0.9 kg of feces and 1.5 kg of urine a day. An average of 36% of the dosed radioactivity was found in the feces and 0.4% in the urine. The residue level in the first week's feces was 555 ppb and analysis indicated 39% of it was parent drug. A reasonable estimate of the total drug equivalent in the first week's feces of swine given ivermectin at the recommended level of 0.3 mg/kg is (0.3/0.4) x 555 ppb = 416 ppb.

The breeder hogs would be dosed with 0.3 mg/kg x 150 kg body weight = 45 mg of drug. If 36% of this dose is

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

also excreted in the first week, the total residue level in the first week's waste would be:

$$\frac{36\% \text{ excreted in first week} \times 45 \text{ mg dose}}{11.6 \text{ kg waste/day} \times 7 \text{ days}} = 0.20 \text{ mg/kg} = 200 \text{ ppb}$$

Since feeder pigs represent 85% of the market, and since the total residue level calculated in feces from feeder pigs (416 ppb) is higher than that calculated in breeder pigs, a level of 416 ppb will be used to calculate the environmental burden from the use of swine manure as fertilizer.

Because nitrogen is often the nutrient that limits plant growth and because nitrogen poses the greatest threat to ground water contamination, it is often used as the basis for determining application rates.[16a] The United States Department of Agriculture recommends the use of no more than 12 tons of fresh animal manure per acre (Appendix 4).

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

Indiana's Cooperative Extension Service recommends application rates of 225 pounds of nitrogen per acre, while Extension Services and Experiment Stations in North Carolina, Iowa, and Maine all recommend application rates based upon soil testing and nitrogen content of the waste.(16a) The Wisconsin Manure Management Plan recommends applying manure at rates which minimize the nitrate-nitrogen accumulation in the soil by using application rates based upon crop removal of nitrogen.(16b) The amount of nitrogen this plan estimates that can be applied without accumulating nitrates is 250 pounds per acre. For swine, with 10 pounds nitrogen per ton of manure (Appendix 4), this would be 25 tons of manure per acre. If 25 percent of the nitrogen is lost prior to application, the maximum rate could be 33 tons per acre. Single applications on a land disposal basis might be two times the annual rate, or 50 tons per acre (500 pounds nitrogen per acre).(16b)

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

In a non-random survey of dairy farmers, twelve percent exceeded 300 pounds nitrogen per acre and seven percent exceeded 400 pounds per acre.(16c) Application of approximately 570 lbs N/acre using swine manure injection into the soil has been reported.(16d) This heavy application was followed by only 80 lbs N/acre in the form of anhydrous ammonia for two years. In a liquid swine manure management system in Texas, settled solids from the liquid manure storage pits were collected, hauled to the field, and injected 10 inches below the soil surface.(16e) Nitrogen loading rates of 600 lbs N/acre/year were used for coastal Bermuda grass, annual hay, oats, and hybrid sudangrass.

Thus, application of 50 to 250 pounds nitrogen (5 to 25 tons of swine manure) per acre is recommended, while 570 to 600 pounds nitrogen (57 to 60 tons of manure)

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

per acre appears to be the highest application rate used for swine wastes. Using swine feces containing 416 ppb of ivermectin at 25 tons per acre would lead to a concentration of 4.0 ppb of ivermectin in the soil, when plowed in to a 6" depth.

$$\frac{416\ \mu g\ residue}{kg\ feces} \times \frac{1\ kg}{2.2\ lb} \times \frac{2000\ lb}{1\ ton\ (U.S.)} \times \frac{1\ mg}{1000\ \mu g} = \frac{378\ mg\ drug\text{-}equivalent}{ton\ (U.S.)\ feces}$$

$$\frac{378\ mg\ residue}{ton\ feces} \times \frac{25\ tons\ feces}{acre} \times \frac{1\ acre}{43{,}560\ ft^2} \times \frac{1000 \mu g}{mg} = \frac{217\ \mu g\ drug\text{-}equivalent}{ft^2}$$

$$1\ ft^2 \times 6"\ deep \times \frac{144\ in^2}{ft^2} \times \frac{16.4\ cm^3}{in^3} \times \frac{1.5\ g}{cm^3\ soil} = \frac{21{,}254\ g\ soil}{in\ 1\ ft^2 \times 6"\ deep\ volume}$$

$$\frac{217\ \mu g\ drug\text{-}equivalent/ft^2}{21{,}254\ g\ soil} \times \frac{1000\ ng}{\mu g} = 10.2\ ng/g\ (ppb)\ drug\text{-}equivalent$$

10.2 ppb drug-equivalent x 39% ivermectin = 4.0 ppb ivermectin in soil

Thus, use of 25 tons swine feces per acre would lead to a soil concentration of parent drug of 4.0 ppb. Use of fertilizer at levels exceeding 25 tons/acre, for example 60 tons/acre, injected 10 inches into the ground, would lead to ivermectin levels of only (60/25)

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

x (6" deep/10" deep) x 4.0 ppb = 5.8 ppb ivermectin in the top 10" of soil.

Where water-washed wastes are used, the amount of ivermectin in the soil will depend upon the amount of dilution water and the spreading rate. Using the figure of 3.5 kg raw waste/day from Figure 1, instead of the 0.9 kg feces/day from the three dosed barrows, the figure of 41 kg/day of water-washed waste from Figure 2, and the spreading rate of 8000 gallons/acre, the level of ivermectin in soil fertilized with water-washed waste would be only 0.42 ppb.

$$\frac{3.5 \text{ kg raw waste} \times 416 \text{ ppb drug-equivalent}}{41 \text{ kg water-washed waste}} = 35.5 \text{ ppb drug-equivalent in water-washed waste}$$

$$\frac{35.5 \text{ µg}}{\text{kg}} \times \frac{1 \text{ kg}}{2.2 \text{ lb}} \times \frac{8000 \text{ gal}}{\text{acre}} \times \frac{7.7 \text{ lb}}{\text{gal}} \times \frac{1 \text{ acre}}{43{,}560 \text{ ft}^2} = \frac{22.8 \text{ µg}}{\text{ft}^2}$$

Using the weight of soil in 1 $ft^2$ by 6" deep from the preceding calculation,

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

$$\frac{22.8 \text{ ug/ft}^2}{21{,}254 \text{ g soil}} = \frac{1000 \text{ ng}}{\mu g} = 1.07 \text{ ng/g} = 1.07 \text{ ppb drug-equivalent}$$

1.07 ppb drug-equivalent x 39% ivermectin = 0.42 ppb ivermectin in soil

These calculated residue levels in fresh feces and water-washed waste represent the "worst-case" situation, since wastes, especially water-washed waste, would be accumulated for longer periods of time than just one week (Appendix 3). This would afford further dilution of the ivermectin in the wastes.

Using the ivermectin levels in the wastes and the Freundlich equation, the ivermectin levels in ground water in contact with fertilized fields can be calculated. The Freundlich equation states that the soil concentration equals a constant times the solution concentration raised to a power, and has the form:

$$\frac{x}{m} = Kc^{1/n}$$

where x/m is the soil concentration in micrograms/gram, c is the solution concentration in micrograms/gram, and

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

K and n are constants. Using the data in Section D.2.(f) Soil Translocation for the desorption of ivermectin from an Iowa clay loam soil, and the soil concentrations determined above, the ivermectin concentration in ground water in fields fertilized at 60 tons/acre with fresh swine feces would only be about 7.8 parts per trillion, a concentration at or below the 48-hour no-mortality level to Daphnia magna. Taking the logarithm of both sides of the Freundlich equation gives:

$$\log (x/m) = \log K + 1/n \log c$$

rearranging to:

$$\log c = n [\log (x/m) - \log K]$$

and using the values for the first desoprtion step of ivermectin from Iowa clay loam soil, K = 255 and n = 1.10, and a soil concentration of 5.8 ppb (0.0058 µg/g), the value of c can be calculated as follows:

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

$$\log c = 1.10 [\log (0.0058) - \log (255)]$$
$$\log c = 1.10x \log (-2.2366 - 2.4065)$$
$$\log c = -5.11$$

Taking the antilogs, $c = 7.8 \times 10^{-6}$ µg/g, or c = 7.8 parts per trillion.

This calculation shows that ground water in contact with soil fertilized at the "worst-case" level of 60 tons feces per acre, using feces collected during the first week post-dose, would not contain ivermectin at levels toxic to Daphnia magna in 48-hour static tests. Use of lower application rates, or water-washed wastes at 8000 gallons/acre, would lead to even lower levels of ivermectin in soil and ground water. The remaining drug-equivalent, though comprising 61% of the feces residue, is considered to be much less toxic than the parent drug and is also not expected to be of environmental concern.

The drug-equivalent and ivermectin levels in the above analysis are quite a bit below the levels of drug and metabolites used in the swine column leaching studies

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

[See Section D.2.(h)3) Toxicity to Aquatic Organisms, Daphnia magna Strauss]. Therefore, even in the "worst-case" analysis, the level of ivermectin in the feces would be so low, that the use of the feces or water-washed waste, as fertilizer would not be an environmental problem.

The ivermectin concentration in the runoff from feedlots can also be calculated using the Freundlich equation. The publication "Development Document for Effluent Limitations Guidelines and New Source Performance Standards for FEEDLOTS - Point Source Category," U.S. Environmental Protection Agency, Washington, D.C. 20460, January, 1974,[13] indicates that dirt lot feedlots contain 50 to 250 hogs/acre. Based upon data already presented, hundred fifty 20 to 27 kg hogs/acre, excreting 0.9 kg of feces a day, would produce 1575 kg of feces a week (250 hogs/acre x 0.9 kg feces/day x 7 days = 1575 kg feces). Of the 416 ppb of

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

total drug residue in the feces, 39% is parent ivermectin. Therefore, a total of 0.256 g of ivermectin would be excreted in the first week (1575 kg feces x 416 ppb x 0.39 = $2.56 \times 10^5$ µg).

Using the Freundlich equation $\frac{x}{m} = Kc^{1/n}$ and mass balance, that is, feces-bound ivermectin plus ivermectin in solution equals total drug, or $(x/m)(m) + (c)(v) = T$, where x = µg drug, m = feces mass in grams, c = solution concentration in µg/ml, T=total drug (µg), V = volume in ml and K and n are constants, the solution concentration of ivermectin in the runoff from a feedlot can be calculated. Solving the mass balance equation for x/m and equating this with the right side of the Freundlich equation gives:

$$\frac{T - cV}{m} = Kc^{1/n}$$

or

$$T = m\,Kc^{1/n} + cV$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

Assuming that none of the drug is adsorbed by soil, and that the Freundlich constants determined for Iowa clay loam soil apply to feces, then the known values are:

$T = 2.56 \times 10^5$ µg ivermectin  $K = 255$

$m = 1.575 \times 10^6$ g feces  $n = 1.10$

With the further assumptions that 4 inches of rain, weighing 411,175 kg/acre, falls on the feedlot, and that two acre-inches (205,500 kg) runs off, then the solution concentration of ivermectin can be calculated with the above equation and values and with $V = 4.11 \times 10^8$ ml. When $c \simeq .0002$ µg/ml, then the calculated value of T equals the amount of ivermectin excreted in the first week's feces, that is $T = (1.575 \times 10^6 \text{ g} \times 255 \times c^{1/1.1}) + (c \times 4.11 \times 10^8 \text{ ml}) \simeq 2.56 \times 10^5$ µg.

This concentration, approximately $2 \times 10^{-4}$ µg/ml, or 0.2 ppb, of ivermectin is the expected solution concentration based upon the above assumptions. If two-acre-inches of water runs off the feedlot, this

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Environmental Burden (Cont'd)

would carry away 41 mg, or 16% of the excreted ivermectin:

205,500 kg runoff x 0.2 ppb = 41 mg drug

$$\frac{\text{41 mg drug in runoff}}{\text{0.255 g drug dosed}} = 16\%$$

Assuming that this runoff all terminates in a lake that constitutes only fourteen to thirty-two times the volume of the runoff volume (1 in 15 to 1 in 33 dilution), the drug concentration would be at the 48-hour Daphnia no-mortality or no-discernible-effect concentration (0.006 to .013 ppb) in the lake.

(b) Metabolism of Ivermectin

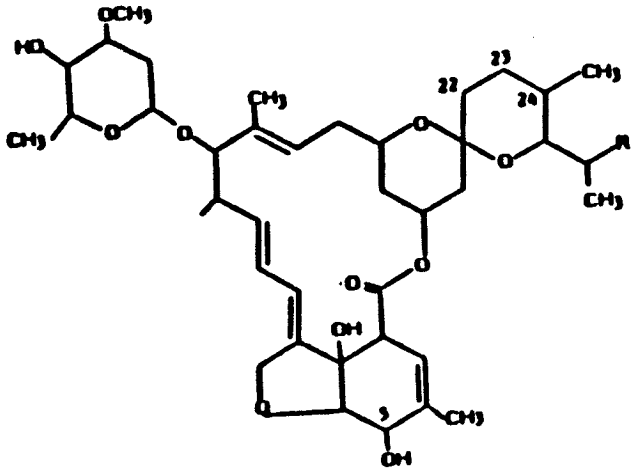
Essentially all of the dosed tritium-labeled ivermectin is excreted via the feces, either as the unaltered drug or as metabolites; less than 1% of the dose was excreted in the urine. Only about 36% of the administered radioactivity was recovered in the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

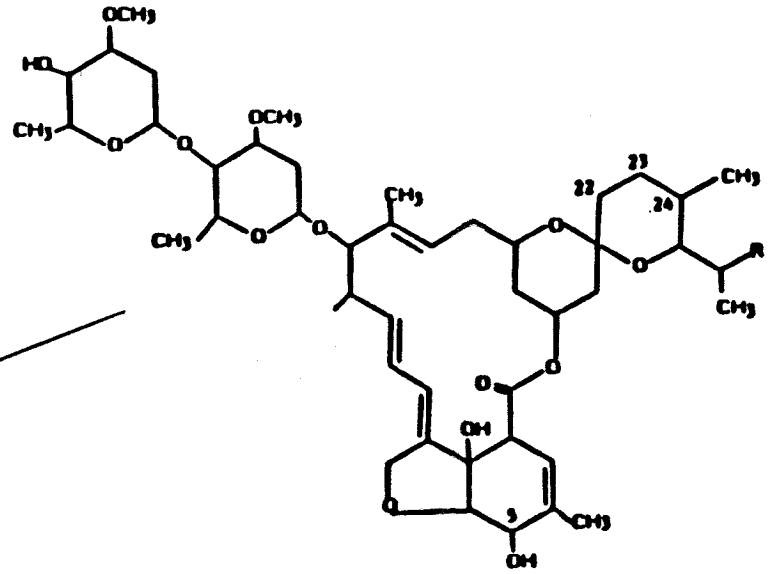
(b) Metabolism of Ivermectin (Cont'd)

excretion during the first 7 days. Analyses of swine feces samples collected during the first 7 days after dosing by the reverse isotope dilution assay accounted for about 39% of the radioactivity in the feces as unaltered drug. The remaining 61% of the radioactivity consisted of ivermectin metabolites. These compounds were moderately soluble in dichloromethane, but are generally more polar than ivermectin. Unlike the liver metabolites of ivermectin in the steer and sheep, which were hydroxylated ivermectins, the monosaccharides and aglycones (see Figure 3), the liver metabolites in the swine were largely the 3"-O-desmethyl-derivatives of the parent drug (see Figure 4). In a composite of 7- and 14-day post-dose swine livers, the parent drug accounted for 45% of the total residue, while 3"-O-desmethyl-$H_2B_{1b}$ and 3"-O-desmethyl-$H_2B_{1a}$ each accounted for 12% of the residue. The major polar metabolites observed in the liver of steers and sheep, the 24-hydroxymethyl-derivatives of the ivermectin

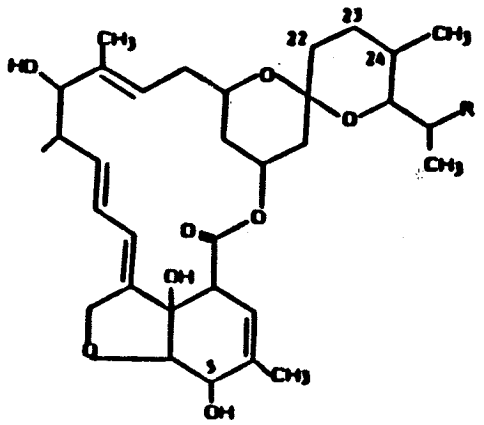
FIGURE 3: PRODUCTS OF HYDROLYSIS OF DIHYDROAVERMECTIN $B_1$



Dihydroavermectin-$B_1$-Monosaccharide



Dihydroavermectin-$B_1$



Dihydroavermectin-$B_1$-Aglycone

Dihydroavermectin-$B_{1a}$: $R = C_2H_5$

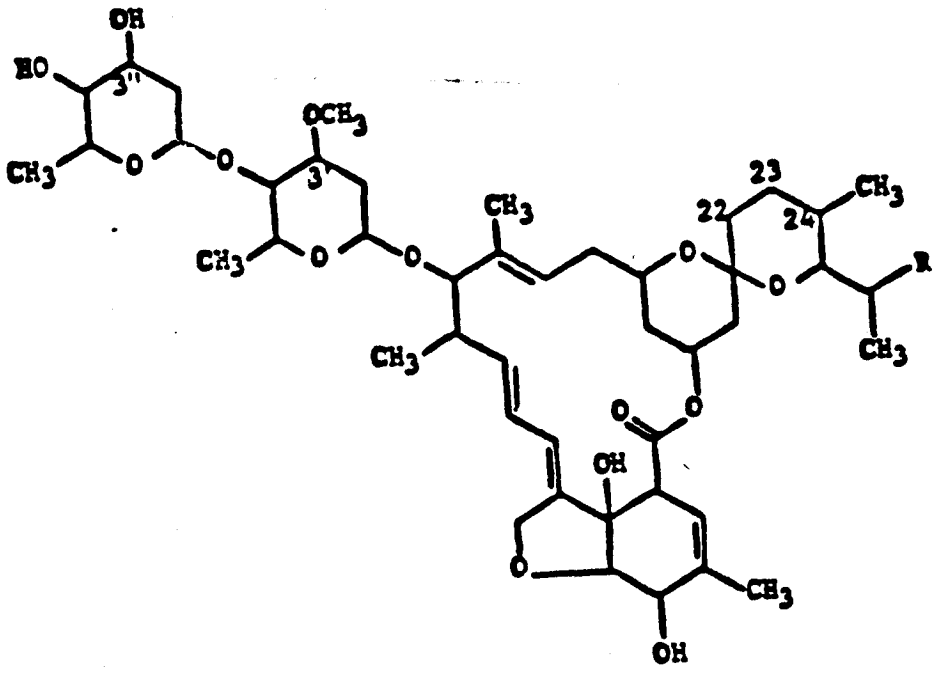
Dihydroavermectin-$B_{1b}$: $R = CH_3$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Metabolism of Ivermectin (Cont'd)

SWINE Liver Metabolites of Ivermectin

Figure 4: Swine Liver Metabolites of Ivermectin




R = $CH_3$, 3"-O-Desmethyl-$H_2B_{1b}$

R = $CH_2CH_3$, 3"-O-Desmethyl-$H_2B_{1a}$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Metabolism of Ivermectin (Cont'd)

components, were present only in trace amounts, if at all, in the swine liver tissue.

A variety of biological assays of several compounds related to ivermectin showed that all these products are less active than the parent drug. Similarly, toxicity of monosaccharide and the aglycone of ivermectin toward Daphnia magna is less than that of parent compound.

Comparison of Dosing and Excretion
of MK-0933 for Swine, Steer and Sheep

| Animal | Dose Route in Study | Dosage Given In Study mg/kg | Dosage Sought In NADA mg/kg | % of Dose Excreted in First 7 Days | | Percent of Parent Drug in Feces |
|---|---|---|---|---|---|---|
| | | | | Feces | Urine | |
| Swine | Subcutaneous | 0.4 | 0.3 | 36 | 0.4 | 39 |
| Steer | Intraruminal | 0.3 | 0.2 | 80 | 0.5 | N.D. |
| Steer | Subcutaneous | 0.3 | 0.2 | 62 | 1.5 | 40-50 |
| Sheep | Intraruminal | 0.3 | 0.2 | 69 | 0.5 | 61 |

N.D. = Not Determined

ENVIRONMENTAL ASSESSMENT (Continued)
IVOMEC (ivermectin) Injection for Swine

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Metabolism of Ivermectin (Cont'd)

Analysis of the feces from a swine dosed with tritium-labeled ivermectin showed that only about 39% of the total fecal residue is intact drug. Thus, 61% of the fecal residue will be drug metabolites. Preliminary uncontrolled studies found that eluates (some diluted) from soil columns containing ivermectin and its metabolites did not solicit a lethal effect in 48 hours on Daphnia.

Comparison of the Toxicity of Ivermectin and the Monosaccharide and Aglycone of its Major Isomer

| Compounds | 48-Hour Daphnia Magna Results | |
|---|---|---|
| | $LC_{50}$, ppb | Approximate No Toxicity Level, ppb |
| MK-933 ($\geq$ 80% $H_2B_{1a}$, $\leq$ 20% $H_2B_{1b}$) | .02 | .01 |
| $H_2B_{1a}$-MS (L638,724) | 0.4 | 0.1 |
| $H_2B_{1a}$-AG (L638,723) | > 17 | 10 |

Chemical structures may be found on page 57.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil

Experiments were conducted to determine the stability of MK-933 in soil and of MK-933 in samples of composite sheep, composite steer, and composite swine feces in soil during the summer. Iowa clay loam soil was used for all samples. This soil had a clay loam texture with a pH of 5.0, was 4.6% organic matter, had a cation exchange capacity of 24.3 meq/100 g, a density of about 1.5 g/cc and a mechanical analysis of 25% sand, 46% silt and 28% clay. The samples were placed outdoors at the Merck site on June 24, 1981.

Percolated rain water was collected as necessary and pooled until time of assay. Assay times were at 0, 1, 2, 4, and 8 weeks. Soil samples were assayed for total radioactivity and for the percent of total radioactivity represented by MK-933. Percolated water samples were assayed for total radioactivity, for extractability into dichloromethane, and for the percent of total radioactivity contributed by MK-933.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

The MK-933 in soil samples were prepared by mixing 1.13 µg MK-933 into 50 g of Iowa clay loam soil, for an MK-933 level of 22.6 ppb. Four 10 g portions were placed on top of glass fiber filters in coarse sintered glass funnels. One 10 g portion was reserved as a zero time sample. The funnels were placed on erlenmeyer flasks in a plastic tub at the outdoor site.

The composite feces in soil samples were all prepared by mixing 2.5 g of the appropriate feces with 47.5 g of Iowa clay loam soil. After thoroughly mixing, one 10 g portion was reserved as a zero time sample, while four 10 g samples were placed in course sintered glass funnels containing glass fiber filters. The funnels were placed on erlenmeyer flasks in the tub at the outdoor site. The feces/soil samples were 50 mg feces/g and total drug equivalent levels were 23 ppb

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

for the composite steer feces in soil 52 ppb for composite sheep feces in soil and 28 ppb for the composite swine feces in soil.

The soil/feces samples were assayed by weighing, homogenizing with an equal weight of water, and combusting triplicate portions to determine total radioactivity per gram. The rest of the homogenate was spiked with unlabeled MK-933 and mixed with an equal volume of acetone. After centrifuging, the acetone was removed and a second acetone extraction was performed. The pooled acetone extracts were evaporated after an aliquot was removed for scintillation counting. The residue was dissolved in methanol, filtered and redissolved in methanol. The MK-933 components were isolated by high performance liquid chromatography (HPLC). The specific activity of the $H_2B_{1a}$ and

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

$H_2B_{1b}$ components of the MK-933 were determined to allow calculation of the percent of MK-933 in the sample.

The rain water which percolated through the samples was collected as needed and frozen until assay time. After thawing, the volumes were determined, aliquots were removed for scintillation counting, and unlabeled MK-933 was added. The aqueous samples were extracted twice with 10% by volume of dichloromethane. The pooled dichloromethane extracts were evaporated after aliquots were removed for scintillation counting and the extracts then chromatographed to determine the percent of MK-933 in the percolates. In all cases, no MK-933 was observed in the aqueous percolates; only very polar compounds were present. The percent of MK-933 in the entire sample (feces/soil mixture plus percolated water) was determined by correcting the percent of MK-933 in the feces/soil mixtures for the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

amount of non-MK-933 radioactivity which percolated. The level of MK-933 in each sample decreased "pseudoexponentially" with half-lives of 1 - 2 weeks in every case. The decomposition products were more polar compounds.

Experiments were also carried out at Merck Sharp & Dohme Research Laboratories (MSDRL) to determine the stability of MK-933 in soil, of MK-933 mixed with control steer feces in soil, of MK-933 and its metabolites in feces from steers dosed with tritiated MK-933 (composite feces) in soil, and of MK-933 in control steer feces when these samples were exposed to an outdoor winter environment. The soil used was the same Iowa clay loam soil previously described. The amount of MK-933 remaining in these outdoor stability samples was determined by reverse isotope dilution assay (RIDA) at various times. Rain water, percolated

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

through these samples, was collected and assayed for dichloromethane- extractable materials, including MK-933, and for toxicity of the eluated materials towards Daphnia magna.

Samples of MK-933 in soil, of MK-933 in control feces in soil, and of composite feces in soil were prepared. Triplicate, weighed portions of each sample were placed into 8 cm coarse-fritted sintered glass funnels. Triplicate samples of MK-933 mixed into control feces were weighed into 4 cm medium-fritted, sintered glass funnels.

The glass funnels were placed into filter flasks and the flasks were placed into a plastic tub and set outdoors in a fenced-in area at MSDRL where the samples were exposed to the outside environment. A graduated cylinder was also placed in the tub to measure rainfall.

The samples were placed outdoors on October 30, 1980.

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Outdoor stability samples were taken after 1 week, 3 weeks, 7 weeks and 13 weeks. Rain water and melted snow were collected as necessary.

Outdoor samples were assayed by adding a known amount of unlabeled MK-933 to a weighed sample. Sufficient acetone was added with mixing to equilibrate the added and endogenous MK-933. Acetone was used to extract the cold carrier and endogenous tritium-labeled MK-933. The extract was chromatographed on a high performance liquid chromatography (HPLC) system. The HPLC eluates which contained $H_2B_{1b}$ and $H_2B_{1a}$ components of MK-933 were collected.

The total radioactivity in the sample was determined by combustion of weighed samples. From the total radioactivity in the extracted sample, the amount of unlabeled $H_2B_{1a}$ and $H_2B_{1b}$ added, and the specific activities of the isolated compounds, the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

percent of each MK-933 component in the sample could be calculated.

Percolated water samples were collected and the total concentration of MK-933, its metabolites and degradation materials in solution was determined by counting aliquots. The amount of radioactivity extractable into dichloromethane was determined either by extracting the original solution, then adding unlabeled MK-933 for RIDA analysis, or by adding an equal volume of acetone and an aliquot of unlabeled MK-933 to the original solution, then extracting with dichloromethane. The material extracted into the dichloromethane was then chromatographed by HPLC, and the specific activities of the $H_2B_{1a}$ and $H_2B_{1b}$ components of MK-933 were determined. The percolated water samples were also assayed for toxicity towards Daphnia magna.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

The dichloromethane extraction and HPLC analysis of the leachate from outdoor stability samples of composite feces in soil indicated less than 10 mg MK-933 per liter in solution. These low levels were confirmed to be innocuous in the Daphnia toxicity experiment, where no mortality occurred in 48 hours. The dichloromethane extractions and HPLC analyses of the leachates from the outdoor stability experiments of MK-933 in soil, of MK-933 in control feces in soil, and of MK-933 in control feces also showed low levels of MK-933 in solution, and less than 50% mortality towards Daphnia magna after 48 hours, in preliminary uncontrolled experiments.

The polar compounds which eluted from the outdoor stability samples of MK-933 in soil represent extensive degradation of MK-933. These compounds were more polar than the corresponding monosaccharides or aglycones of MK-933. The amount of these polar decomposition materials which percolated must be included with the

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

amount of polar decomposition materials in the soil samples when determining the rate of degradation of the MK-933 in soil. Initially, MK-933 comprised 59% of the radioactivity in the MK-933 in soil sample and polar compounds accounted for only 2%. After 13 weeks, about 17% of the initial radioactivity had eluted as polar material. Inclusion of this eluted polar material with the polar compounds in the soil means that 46% of the radioactivity after 13 weeks was polar material, while 31% of the total radioactivity was MK-933. Thus, in 13 weeks, the level of MK-933 decreased from 59% to 31% indicating a half-life of about 14 weeks.

Similarly, for the outdoor sample of composite feces in soil, 35% of the original radioactivity percolated in 13 weeks, and the decrease in remaining MK-933 in the sample suggested a half-life of about 31 weeks. For MK-933 in control feces in soil, the half-life estimate was 52 weeks, and for MK-933 in control feces without soil, it was 16 weeks.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Thus, there were low levels of MK-933 in the leachates from the outdoor winter stability samples of MK-933 in soil, MK-933 in control feces in soil, MK-933 in control feces without soil, and of composite feces in soil. The levels of MK-933 in these were below $LC_{50}$ level of 20 ng/l as determined by Daphnia magna assay. Most of the percolated radioactivity represented highly polar material, indicating extensive degradation of the original MK-933. Half-life estimates for the degradation of MK-933 in various soil/feces mixtures in an outdoor environment from November through January ranged from 14 weeks to 52 weeks.

Additional experiments on the stability of ivermectin in feces mixed with soil were carried out in aerated brown bottles at 22°C over a period of 3 to 168 days. The experimental set consisted of two types of samples: (1) feces from a steer dosed with tritium-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

labeled ivermectin and (2) control feces spiked with tritium-labeled ivermectin. Each of these materials was mixed with either sandy loam pasture or gravelly clay forest soils. These soils were from Clayton, NC, the sandy loam pasture soil was comprised of 74% sand, 18% silt and 8% clay, while the gravelly clay forest soil was 60% sand, 27% silt and 13% clay. The experiments were set up with triplicate samples. On a prescribed schedule, the samples were extracted with water and then water/ acetone; the extracts and spent solutions were assayed for radioactivity content; and the water/ acetone extract assayed by an HPLC/fluorometric procedure for the ivermectin content.

The detection limit for the radioactivity measurement was about 1 ppb and for the fluorescence assay about 10 ppb. The concentrations of ivermectin and metabolites in the stability, percolation and other soil experiments were set high enough to permit assay of the feces/soil samples.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Considerable intragroup and intergroup variation in the radioactivity of ivermectin assays was encountered. Calculations of the half-life for the degradation process were by the linear least squares fitting of the logarithm of concentration at sample time. The half-lives indicate a relatively slow, but significant, degradation of ivermectin. Thus, there would not be a gradual accumulation of ivermectin even if it were introduced into the soil on repeated but infrequent intervals.

Half-lives of Ivermectin Degradation in Feces/Soil Mixtures

| Sample | Soil | Half-life-days |
|---|---|---|
| Feces from dosed animal | Pasture | 196 |
| Feces from dosed animal | Forest | 111 |
| Control feces plus ivermectin | Pasture | 169 |
| Control feces plus ivermectin | Forest | 260 |

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

Most of the degradation products, which amounted to 30-50% of the drug in all samples at 168 days, were found to be extractable into non-polar solvents but did not respond to the chemical assay for ivermectin.

Although the stability studies were designed to yield information on the rate of decomposition, it is difficult to extract a definitive rate covering the entire 24-week period of the study. Considering the data for the $B_{1a}$ component, the rate of disappearance appears much faster in the early periods. Application of a two-compartment model allows the estimation of an early period half-time (up to 14 days) and a terminal halflife (28-168 days). By this procedure, the rate of disappearance in the early period corresponds to a half-life of 2.6-5.8 days. During the terminal part of the experiment, half-life estimates vary from about 139 to 365 days. This interpretation is consistent with

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Stability of Ivermectin in Soil (Cont'd)

the hypothesis that the rate of degradation by microorganisms is not the rate-limiting step in either of the two phases. The use of the two-compartment model is consistent with the observation that ivermectin is very strongly absorbed on soil. In the strongly adsorbed state, as represented by the terminal phase of the experiment, the rate of desorption from the soil is the rate-limiting step. The rapid half-life observed in the earlier period reflects the faster desorption from the fecal material. High concentrations of ivermectin are available for microbial action during the time that the ivermectin is equilibrating from the fecal matter to the higher-affinity sites in the soil.

At the end of the experiment, a small fraction (2.4-7.0%) of the initial radioactivity had been collected in the ethanol used to trap volatile products. The formation of such products indicates that extensive degradation of the ivermectin and metabolites had occurred.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Stability of Ivermectin in Aqueous Extracts of Steer Feces

To simulate the situation expected to arise in runoff from feedlots containing ivermectin-treated cattle, the extractability of ivermectin and its metabolites from fecal material by water, and the stability of ivermectin in the aqueous phase, were studied.

Samples of feces from steers dosed with tritium-labeled ivermectin were extracted with either lake water or reverse osmosis water at a level of 50 mg of feces/ml of water. The feces used in the study were a composite collected 2 to 5 days after a 0.3 mg/kg dose, and the level of ivermectin and its metabolites was 600 ppb.

After blending, centrifuging and filtering through glass fiber filters, the fecal extracts were transferred to 1000-ml amber bottles, sealed with rubber septa and stored at 22°C. Samples were flushed three times a week with dry air to prevent anaerobiosis. Weight losses caused by evaporation

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Stability of Ivermectin in Aqueous Extracts of Steer Feces (Cont'd)

during flushing were corrected by addition of reverse osmosis water. Samples were assayed at 0, 2, 7, and 11 days.

At a loading of 50 mg feces/ml water, 30 ppb would be the maximum nominal concentration of ivermectin and metabolites in the extracts. Based upon the tritium-activity, lake and reverse osmosis water extracted 36.5 and 35.0%, respectively of the total radioactivity, for solution concentrations of about 10-12 ppb in total drug-equivalent. High performance liquid chromatographic analysis determined the concentration of ivermectin in lake water at 2.8 ng/ml (ppb) and at 2.6 ng/ml in reverse osmosis water, which are 9.3% and 8.7% of the maximum nominal concentrations, respectively. Comparison of mean tritium-activity in the extracts and mean ivermectin concentration between lake and reverse osmosis water showed no significant differences. Also, there was no significant decline in the ivermectin or

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Stability of Ivermectin in Aqueous Extracts of Steer Feces (Cont'd)

total residue level during 11-day study in either lake or reverse osmosis water.

The small amount of unaltered drug extracted from the feces into the water probably reflects sorption to the organic material in the cattle wastes.

(e) Stability of Ivermectin in Water and Water/Swine Feces Homogenate

Since many swine are raised in solid concrete-floored facilities which generate water-washed wastes, an experiment was run at MSDRL to determine the stability of ivermectin in water and in a water/swine feces homogenate. The water/feces homogenate was prepared in a similar ratio as expected under feedlot conditions. The stability of MK-933 in pure water was also examined to compare with the stability of MK-933 in the water/swine feces homogenate.

Samples were prepared using filtered, distilled water

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Stability of Ivermectin in Water and Water/Swine Feces Homogenate (Cont'd)

and sterilized amber bottles. For the swine feces/water homogenate, a sample of 22 g of composite feces was homogenized with 228 g of water. Ten 25 g samples were placed into bottles which were capped and wrapped in foil to exclude light. The drug-equivalent and ivermectin levels were 49 and 19 ppb, respectively. For the MK-933 in water, tritium-labeled and unlabeled MK-933 were mixed and homogenized in 250 ml water. Ten 25 g samples were bottled, capped and wrapped in foil to exclude light. The MK-933 level was 110 ppb.

At assay times of 0, 2, 7, 114, 28 and 42 days, 1 or 2 samples were taken, and cold carrier MK-933 was added along with acetone. After mixing and reducing the volume of acetone, pH 7 buffer and cyclohexane were added and shaken. The cyclohexane was removed, filtered and blown dry under nitrogen. The residue was resuspended in methanol and chromatographed to isolate the $H_2B_{1b}$ and $H_2B_{1a}$ components of the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(e) Stability of Ivermectin in Water and Water/Swine Feces Homogenate (Cont'd)

ivermectin. The specific activities of the MK-933 components were measured to determine the percent of the total radioactivity comprised by MK-933.

Within the accuracy of the determination, ivermectin decomposed very little, if at all, in either pure water or the water/swine feces homogenate at room temperature in the dark.

A soil translocation (leaching) study with excreta from cattle injected with radioactive ivermectin was conducted with four different soil types.

These soils were a silt loam from Ambler, Pennsylvania, a clay loam from Newton, Louisiana, a loam from Greenville, Mississippi and a sandy loam from Clayton, North Carolina.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation

Textural analysis (%) of these soils is as follows:

| | Sand | Silt | Clay |
|---|---|---|---|
| Ambler, PA, Silt Loam | 22 | 55 | 24 |
| Newton, LA, Clay Loam | 22 | 48 | 31 |
| Greenville, MS, Loam | 52 | 34 | 15 |
| Clayton, NC, Sandy Loam | 74 | 18 | 8 |

(1) The soil leaching study was designed to determine the movement and distribution of a test material and metabolites through a 30 cm soil column. Columns were prepared in triplicate for each soil type. The feces (0.5 g) was mixed with 1 g soil and applied to the top of the column. Water, corresponding to 10-20 acre inches, was applied and 180-600 ml of leachate was collected, depending on soil type. The columns were cut in seven segments, and radioactivity measured (after combustion) by liquid scintillation.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

The mean radiolabel recovered in the leachates was:

| | Volume ml ± Std. Dev. | Total % Eluted | % per ml of leachate |
|---|---|---|---|
| Silt Loam | 295 ± 37 | 26.98 ± 5.56 | 0.091 |
| Clay loam | 595 ± 5 | 48.07 ± 1.54 | 0.081 |
| Loam | 193 ± 14 | 9.83 ± 1.67 | 0.051 |
| Sandy loam | 591 ± 3 | 42.73 ± 10.0 | 0.072 |

The radioactivity in the effluent was subjected to extraction with methylene chloride, which was expected to remove ivermectin and metabolites. More than 87% of the radioactivity was extracted (the loam effluent was not processed), but HPLC analysis showed no unchanged ivermectin (detection limit 13 ± 7 ng or 4% of the amount charged to the columns). Activity in the effluents is probably due to metabolites but is not unchanged ivermectin.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

Analysis of segments of the soil column for total radioactivity showed that most of the remaining radioactivity was within the top 2.5 cm of the column and hence had not been present in the effluent.

(2) To simulate pasture conditions a similar soil leaching was run, using specimens prepared by mixing 5 grams of feces (in suspension) with 50 grams of sandy loam soil in replicate, and storing the material aerobically at 22°C for 30 days. This aerobically aged feces/soil material was then added to the top of sandy loam columns and leached slowly (15 ml per day) for 45 days. The diffusion of activity in the soil was similar as for the unaged material: 46.83 ± .74%, eluted corresponding to 0.074% per ml.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

In all cases, including the aged specimens, a large portion of the applied activity remained in the top segment of the soil columns. This gave additional evidence that the material is not likely to be readily translocated into ground water.

It is known from an isotherm experiment that soil contains high affinity sites for ivermectin and that soil has a relatively high capacity for ivermectin. Even in the presence of trace amounts of DMSO (used to dissolve ivermectin), soil has been shown to decrease the level of ivermectin in solution below the $LC_{50}$ of Daphnia (about 20 ppt).

Another experiment which indicated ivermectin would not translocate in soil was an isotherm study conducted with Iowa clay loam soil. This soil has a pH of 5,

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

4.6% organic matter, cation exchange capacity of 24.3 meq/100 g, density of 1.5 g/cc, and a mechanical analysis of 26% sand, 46% silt and 28% clay.

A preliminary study was performed to determine the length of time necessary for an equilibrium to take place between this Iowa soil and ivermectin. Sixteen hours was determined to be sufficient and this period of time was used in the subsequent ivermectin sorption/desorption test phases.

Sorption/desorption of tritium-labeled ivermectin in Iowa clay loam was measured as follows:

Centrifuge tubes with plastic caps were individually tared and 7.5 g of 0.01 M $CaCl_2$ solution and 1.5 g air-dried Iowa soil was added to each. A stock solution of 0.463 mg/ml of tritium-labeled ivermectin in methanol was aliquotted directly into the tubes containing soil and water. Triplicate tubes were

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

treated with 10, 25, 50, or 100 µl of stock ivermectin solution, receiving 4.6, 11.6, 23.2, and 46.3 µg ivermectin, respectively (corresponding to nominal solution concentrations of 0.62, 1.55, 3.1, and 6.2 ppm). After mixing, the sorption aqueous phase ivermectin concentrations were measured directly via scintillation counting, and the sorption soil ivermectin concentrations were measured (in duplicate) via combustion (of a small soil sample), tritium trapping, and scintillation counting. Two successive desorption phases followed in which a fresh $CaCl_2$ solution was added to the remaining soil samples, was mixed for 16 hours, and a new equilibrium was established. For both desorption phases, the aqueous and soil concentrations of ivermectin were determined as in the prior sorption phase of the study.

The respective sorption and two desorption values for K (strength of sorption or desorption) were estimated to be 176, 255, and 238 with respective values of n =

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

1.17, 1.10, and 1.13, where bound ivermectin concentration = K x (solution concentration)$^{1/n}$. The sorption $K_D$ values (distribution sorption coefficient = µg drug/g soil ÷ µg drug/g solution) decreased in a direct fashion with increasing drug concentration. The average sorption $K_D$ values were 332, 307, 262, and 231 over the dose range of 0.62, 1.55, 3.1, and 6.2 ppm, respectively. Calculation of the respective $K_{oc}$ values ($K_{oc} = K_D \times 100/\%$ organic carbon, where % organic carbon = % organic matter/1.724) for the above ivermectin dose range resulted in $K_{oc}$ values of 12,400, 11,500, 9,800, and 8,650. The decreasing $K_D$ and $K_{oc}$ values clearly imply that increased soil sorption of ivermectin occurred as the solution ivermectin dose level decreased.

Compounds having $K_{oc}$ values of $\geq$ 1,000 are tightly bound to the soil organic matter and these compounds can be considered to be immobile in that soil. The

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

data from this study, therefore, supports the conclusion that ivermectin is tightly bound to Iowa clay loam soil and would not translocate.

Further corroboration of the lack of soil translocation of ivermectin comes from soil TLC and soil column studies with the related compound, avermectin $B_{1a}$.

The experimental conditions for the soil TLC method were as follows: All soils were air dried and sieved prior to use, the six soils used were Lufkin sandy loam, Lakeland sand, Houston clay loam, Three Bridges silt loam, Riverside loam, and Samford sand. The soils ranged in pH from 5.6 to 7.5, in organic matter from 0.1 to 4.8%, in C.E.C. from 1.5 to 39.2 m equiv./100 g, in $H_2O$ retention from 1.2 to 34.7%, in sand from 11.6 to 95.6%, in silt from 1.6 to 61.6%, and in clay from 2.8 to 30.8%. A slurry of soil, water, and $CaSO_4$ was made and spread onto 20 x 20 cm glass plates. The

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

plates were air dried at room temperature and four $^{14}C$-labeled pesticide standards (2,4-D, parathion, aldicarb and Mirex) were applied to the plates side-by-side, along with $^{14}C$-avermectin $B_{1a}$, and then developed with water (about 15 cm) via ascending chromatography. The plates were visualized by autoradiography with x-ray film. Aldicarb and 2,4-D were generally very mobile, with Rf values usually greater than 0.6. In contrast, Mirex, parathion, and avermectin $B_{1a}$ were all immobile in water, with Rf values of 0 in all six soil types.

The soil column leaching of $^{3}H$-avermectin $B_1$ in four soils (Lakeland sand, Lufkin sandy loam, Houston clay loam, and Three Bridges silt loam) was evaluated by first packing about 38 cm of sieved soil into six glass columns (4 x 52 cm) of each soil, applying 10 micrograms of the labeled pesticide to the soil at the top of each column (except for the controls), adding 2 cm of additional soil to each column and then wrapping each column in aluminum foil to prevent light exposure. For each soil type, two

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

treated and one control columns were allowed to age for 29 days at room temperature prior to water application. Both unaged and aged columns received about 760-800 ml of water during a 28-day period (at a rate of about 50-100 ml every 2-3 days). This amount of water was equivalent to a total of ca. 22-23" of rainfall. The leachate of each column was collected and radio-assayed using liquid scintillation. After leaching, the soils in the columns were sectioned into 6 cm segments, air dried and radio-assayed via combustion analysis. Most of the radioactivity remained in the top 6 cm of soil (ca. 83-92% in the unaged columns and ca. 79-89% in the aged columns). This indicated that avermectin in $B_{1a}$ absorbs tightly on all soil types. The small amounts of radioactivity found in the rest of the soil column (up to 13%) and in the leachates (up to 7.8%) were probably due to the channeling effect in the column packing. Little difference was seen in aged versus unaged results.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

Solvent extractions and HPLC/radio-assay of the residues in the top 6 cm soil segments indicated that (depending on the soil type) significant amounts of avermectin could be transformed into either more polar compounds and/or became unextractable from the soil. The Lakeland sand showed the lowest remaining percent of parent avermectin, with concomitant high residues of polar metabolites and unextractable radioactivity. The polar metabolites of avermectin $B_{1a}$ resulted from degradation by the soil microbes.

An extraction and assay of the avermectin in $B_{1a}$ in the column leachates (left stored at ambient temperatures for about three months) indicated that usually all of the radioactivity existed as polar metabolites of avermectin $B_{1a}$.

Thus, in the soil thin-layer methods, avermectin $B_{1a}$ was determined to be "immobile" in all six soil types.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(f) Soil Translocation (Cont'd)

In the soil column leaching test, avermectin $B_{1a}$ tended to stay in the top 6 cm of the soil columns. A small percentage of the remaining radioactivity was found to reside in the leachate and also in some of the other soil column segments. Avermectin $B_{1a}$ was also determined to be "immobile" in the soil column leaching studies.

(g) Soil Toxicity-Microbial Effects

A laboratory screening test was conducted to determine the potential for ivermectin residues present in wastes from treated cattle to affect two soil processes: the microbial conversion of soil ammonia to nitrate (nitrification) and the overall conversion of carbonaceous soil organics to carbon dioxide (soil microbial respiration). Other soil microbial community processes and activities were not examined.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Soil Toxicity-Microbial Effects (Cont'd)

In pasture and forest soils to which approximately 5% feces from a steer treated with ivermectin was added, no biologically significant effects on nitrification or overall soil community respiration were observed during the one-month test period when those results were compared to control soils amended with steer feces that did not contain ivermectin residues. The 5% amendment rate was approximately two and one half to ten times the rate normally used when cattle manure is applied to agricultural soils as a fertilizer. No other amendment levels or ivermectin doses were screened in this test.

(1) Nitrification

The effect of steer feces containing ivermectin and its metabolites on the nitrification process in two types of soil was determined by measuring their effect on the reaction.

$$NH_4^+ \longrightarrow NO_2^- \longrightarrow NO_3^-$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Soil Toxicity-Microbial Effects (Cont'd)

An aqueous homogenate of steer feces was added to either pasture or forest soil at a level of 50 mg feces/g soil (or 30 ppb of ivermectin plus metabolites).

The following is the soil characteristics for these test soils:

| Soil | Pasture | Forest |
|---|---|---|
| Soil Type | Norfolk Sandy Loam | Gravelly Clay |
| Texture | Sandy Loam | Sandy Loam |
| Percent Organic Matter | 4.26 ± 0.17 | 6.48 ± 0.02 |
| Water Capacity (g $H_2O$/g soil) | 0.2980 | 0.3805 |
| Percent of Saturation | 26.58 | 23.23 |
| pH | 5.21 ± 0.03 | 4.31 ± 0.02 |

After the mixture aged for periods from 0 to 4 weeks, $(NH_4)_2SO_4$ at a level of 100 ppm N was added and the system further aged for 1-2 weeks. The samples were extracted and the concentration of ions in solution measured by the use of ion-specific electrodes. Sodium azide ($NaN_3$) at a concentration of 1000 ppm was used as a positive control.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Soil Toxicity-Microbial Effects (Cont'd)

The data summarized in Table 2 show a very small, variable effect of ivermectin on the nitrification process in soil as indicated by the measured ion concentrations. The positive control $NaN_3$, at 2 and 4 weeks consistently showed reduced concentrations of $NO_3^-$ and increased concentrations of $NH_4^+$ and $NO_2^-$ compared with the controls.

Even though there is essentially no effect of ivermectin and its metabolites on the nitrification process in soil at the level of 30 ppb, the actual concentrations in soil of plowing in cattle manure (assuming a mixture down to 6 inches), is about 0.1 ppb or a factor of 300-fold lower.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Soil Toxicity-Microbial Effects (Cont'd)

Table 2: Effect of Ivermectin and Metabolites in Feces on Soil Nitrification Process

| Assay Weeks | Soil Type | Treatment | Mean - ppm N in Soil $NO_3^-$ | $NH_4^+$ | $NO_2^-$ |
|---|---|---|---|---|---|
| 0 | Pasture | Control Feces | 42 | 35 | 10 |
| | | Feces + drug | 51 | 46 | 9 |
| | | $NaN_3$ 1000 ppm | 41 | 94 | 9 |
| 0 | Forest | Control | 14 | 85 | 0.5 |
| | | Feces + drug | 14 | 101 | 0.5 |
| | | $NaN_3$ | 14 | 119 | 13 |
| 2 | Pasture | Control | 85 | 9 | 2 |
| | | Feces + drug | 120 | 5 | 1 |
| | | $NaN_3$ | 30 | 100 | 12 |
| 2 | Forest | Control | 38 | 73 | 2 |
| | | Feces + drug | 43 | 92 | 2 |
| | | $NaN_3$ | 25 | 141 | 26 |
| 4 | Pasture | Control | 127 | 10 | 6 |
| | | Feces + drug | 155 | 7 | 6 |
| | | $NaN_3$ | 51 | 115 | 11 |
| 4 | Forest | Control | 43 | 69 | 1 |
| | | Feces + drug | 33 | 71 | 1 |
| | | $NaN_3$ | 18 | 145 | 19 |

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Soil Toxicity-Microbial Effects (Cont'd)

(2) Respiration

The effect of steer feces containing ivermectin and its metabolites on the respiration process in two types of soils was determined by periodically measuring the $CO_2$ content in the head gas of the bottles containing the feces-soil mixture.

The steer feces had no effect on respiration compared to the controls in pasture soil and caused only a very small increase in respiration in forest soil. Sodium azide clearly depressed respiration in both soils. The data are summarized in Table 3.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Soil Toxicity-Microbial Effects (Cont'd)

Table 3: Effect of Ivermectin and Metabolites in Feces on the Soil Respiration Process

| Assay Days | Soil Type | Mean Accumulation of % $CO_2$ | | |
|---|---|---|---|---|
| | | Control | Feces | Sodium Azide |
| 1 | Pasture | 1.9 | 1.8 | 1.1 |
| | Forest | 3.5 | 3.1 | 1.6 |
| 2 | Pasture | 3.0 | 2.7 | 1.4 |
| | Forest | 5.3 | 4.7 | 2.1 |
| 6 | Pasture | 6.9 | 6.1 | 1.9 |
| | Forest | 10.4 | 9.9 | 3.0 |
| 10 | Pasture | 10.8 | 10.1 | 2.2 |
| | Forest | 15.7 | 15.7 | 3.5 |
| 20 | Pasture | 19.4 | 17.6 | 2.9 |
| | Forest | 27.8 | 30.2 | 4.6 |
| 30 | Pasture | 24.3 | 21.5 | 3.2 |
| | Forest | 34.2 | 37.8 | 5.1 |

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(g) Soil Toxicity-Microbial Effects (Cont'd)

As with the nitrification experiment, the test level of 30 ppb is a factor of 300-fold higher than that which would be expected in a field fertilized with manure from a cattle feedlot (at 5 tons/ acre) and plowed to a depth of 6 inches (a calculated concentration of about 0.1 ppb).

(h) Phytotoxicity of Ivermectin

Chlorella pyrenoidosa, a fresh water unicellular, non-motile chlorophyte, was used in an algal assay bottle test to determine the toxicity of ivermectin toward algae. The experiment was carried out by preparing a stock solution of ivermectin in N,N-dimethylformamide (DMF) at a concentration of 20 mg/ml. The test concentrations were prepared by mixing the required volume of stock solution with synthetic algal nutrient medium to yield the appropriate final concentration.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

A medium control, a solvent control (0.5 ml DMF/l) and ivermectin test concentrations of 1.0, 1.8, 3.2, 5.6, and 10.0 mg/l (ppm) were prepared.

The tests were carried out in Erlenmeyer flasks, continuously agitated under fluorescent lighting and maintained at 24°C. Cell counts were made on 0, 2, 3, 4, 7, 9, 11, and 14 test days. The following results were obtained:

(1) Effect on overall cell growth: None

(2) Effect on μmax (mean specific growth rate): None

(3) Effect on Maximum Standing Crops (MSC), cell/ml: Significantly reduced when compared to controls.

(4) Effect on algal biomass: Significantly reduced in the 10.0 mg/l concentration.

(5) Effect on lag period: None

It is obvious from these results that ivermectin, at these relatively high concentrations, has moderate effect on the growth characteristics of this alga.

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

A considerable volume of data has been generated on three ivermectin analogs, avermectin $B_1$, avermectin $B_{2a}$, and avermectin $B_{2a}$ 23-ketone, of interest in plant agriculture which demonstrates the complete lack of phytotoxic effects at application rates as high as 9.0 lb active ingredient per acre. A summary of the studies follows:

To date the three avermectin compounds of interest to row crop agriculture, avermectin $B_1$, $B_2$, and $B_2$ 23-ketone, have been evaluated on plants in more than 50 greenhouse trials and more than 30 field trials on a total of 17 crops. There has been one as yet unexplained incident of phytotoxicity with avermectin in a cooperator trial on tomatoes at Bradenton, Florida. In the remaining approximately 80 greenhouse and field studies, no phytotoxicity nor other adverse effects on plant growth have been observed due to avermectin treatment.

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

Alfalfa: Foliar applications of avermectin $B_1$ to alfalfa at rates as high as 0.1 lb ai/acre in four field trials in 1980 resulted in no observable phytotoxicity.

Apples: Eighteen field studies have been conducted with avermectin $B_1$ on apples during the years 1979 and 1980. The highest rate was 0.02 lb ai/100 gallons (approximately 0.16 lb $B_1$/acre) applied to foliage in dilute spray. No phytotoxicity was recorded. A single case of phytotoxicity was observed when avermectin $B_1$ was combined with oil and followed three days later with an application of fungicide (Captan). The injury was similar to that caused by the interaction of oil and Captan on apple foliage, and the investigator attributed the phytotoxicity to the oil/Captan combination and not to avermectin $B_1$.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

Cabbage: Two trials were conducted with avermectin $B_1$ as foliar application on cabbage in 1980. The highest use rate was 0.05 lb ai/acre.

No phytotoxicity was observed. In soil incorporation tests conducted in 1979, avermectin $B_1$ and $B_{2a}$ at rates of 2.5 lb active/acre did not adversely affect cabbage growth.

Collards: A single trial was conducted at 0.05 lb $B_1$/ acre. No phytotoxicity was observed.

Corn: A single greenhouse and one field trial have been conducted on corn. In the greenhouse study no phytotoxicity was observed with avermectin $B_1$ or avermectin $B_2$ when soil incorporated at 1.5 lb ai/ acre. In the field trial, no phytotoxicity was observed with multiple applications of 0.05 lb ai/acre of avermectin $B_1$ to corn foliage.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

Cotton: Seven field trials have been conducted on cotton. Multiple applications of 0.05 lb ai/acre and a single application of 0.1 lb ai/acre resulted in no observable phytotoxicity with avermectin $B_1$.

Cucumbers: Several greenhouse trials have been conducted with avermectin $B_1$ and avermectin $B_2$. Single applications of these materials to soil at rates as high as 9.0 lb ai/acre resulted in no observable phytotoxicity.

Grapefruit: Field trials have been conducted with avermectin $B_1$ in 1979 and 1980 at rates as high as 6 ppm (0.04 lb $B_1$/acre) in dilute sprays. No phytotoxicity has been observed.

Lima Beans: Approximately 25 greenhouse trials have been conducted with avermectins on lima beans. This has been the major greenhouse screening plant for foliar miticidal activity. No phytotoxicity has been

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

observed at any rate on foliage or in the soil with any avermectin or avermectin formulation on this plant species.

Oranges: Field trials have been conducted with avermectin $B_1$ at rates as high as 25 ppm (approximately 0.16 lb $B_1$/acre) applied to foliage and fruit. No phytotoxicity has been observed.

Peaches: One field trial was established on peaches in 1980. A single application of 16 ppm (approximately 0.10 lb $B_1$/acre) of avermectin $B_1$ in dilute spray results in no phytotoxicity symptoms.

Pears: Three field trials were conducted on pears during 1980. Rates of avermectin $B_1$ as high as 16 ppm (ca. 0.10 lb $B_1$/acre) in dilute spray were non phytotoxic to fruit or foliage.

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

Peanuts: A single field trial was conducted on peanuts during 1979 with avermectin $B_{2a}$. No phytotoxicity or adverse plant growth was observed at rates as high as 1.35 lb $B_{2a}$/acre incorporated into soil.

Potatoes: Five field trials were conducted on potatoes during 1980. Multiple foliar applications of avermectin $B_1$ at rates as high as 0.05 lb ai/acre did not cause phytotoxicity.

Sweet Corn: Three field trials were conducted on field corn during 1980. Multiple spray applications of avermectin $B_1$ at rates as high as 0.05 lb ai/acre did not cause phytotoxicity.

Tobacco: During 1979 and 1980 five field trials were conducted with avermectin $B_1$, $B_2$ or $B_2$ 23-ketone incorporated into soil at 0.45 lb active/acre. No sign of phytotoxicity was observed under a variety of soil conditions.

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2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(h) Phytotoxicity of Ivermectin (Cont'd)

Tomatoes: Several greenhouse trials have been conducted with avermectin $B_1$ and/or avermectin $B_2$. Rates as high as 9.0 lb ai/acre of these materials incorporated into the soil did not result in phytotoxicity.

Three field trials were conducted with the avermectins on tomatoes at the same location in Bradenton, Florida. In the first two trials no phytotoxicity was observed when soil incorporated at rates as high as 3.0 lb ai/acre for avermectin $B_1$ and avermectin $B_2$ and at 1.0 lb ai/acre of avermectin $B_2$ 23-ketone. However, in the third trial conducted under similar conditions by the same researcher slight stunting was reported with $B_1$ when incorporated in soil at 0.3 lb ai/acre.

It is believed that the rate projected from applications of swine manure, would have no phytotoxic effect on naturally occurring plant species.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism

(1) Bluegill Sunfish

Bluegill sunfish, Lepomis macrochirus Rafinesque, a warm-water fish of wide geographic distribution and important as a food-web organism, is recommended as a bioassay organism. Range finding tests were carried out with 4 liters of water to which had been added suitable aliquots of ivermectin dissolved in N,N-dimethylformamide (DMF). A sample of pure dilution water and also one containing DMF (as in the highest test concentration) were used as controls. Four test organisms were added to each solution and mortalities were recorded at 24, 48, 72 and 96 hours.

The definitive test was carried out in 15 liters of water contained in a 19.6 liter glass jar.

Ivermectin dissolved in DMF was added to the water to give concentrations of 5.6, 10.0, 18.0, 32.0

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

and 56.0 µg/l. Pure dilution water and water containing DMF at the highest concentration used in the test served as controls.

Ten bluegill sunfish, approximately 4 months old and 35 mm in length, were introduced into each test and control jar maintained at 20-21°C and the mortality and abnormal behavior of the fish observed at 24, 48, 72 and 96 hours.

The 96-hour $LC_{50}$ (with 95% confidence limits) for ivermectin was 5.3 (4.4 - 6.4) µg/l.

(2) Rainbow Trout

Rainbow trout, Salmo gairdneri, prefers water temperatures below 20°C, has a wide geographic distribution and occupies an important place in the aquatic food web. For these reasons, the rainbow trout is recommended as a bioassay test organism.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

The assay was carried out in the same manner as that described for the bluegill sunfish except that the age of the trout was approximately 3 months, the mean length about 45 mm, and the temperature of the water 11.5-12°C. The 96-hour $LC_{50}$ (with 95% confidence limits) for ivermectin was 3.3 (2.8-4.0) µg/l.

(3) Daphnia magna Strauss

Daphnia magna Strauss, because of its wide geographic distribution and importance in the foodweb, is recommended as a bioassay test organism.

Two laboratory studies were conducted to determine the toxicity of ivermectin towards Daphnia. In the range-finding test for the first study, suitable aliquots of a solution of ivermectin in DMF (1.0 mg/ml) were added to 500 ml of dilution water. The diluted solution was thoroughly mixed

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

and divided equally in two replicate polypropylene beakers. Fourteen concentrations were tested, plus a water control and a water/solvent control, containing the same DMF concentrations as in highest range-finding concentrations. Five newly released instar daphnids, less than 20 hours old, were carefully added to each beaker of test solution and controls. Mortalities were recorded at 24 and 48 hours.

The definitive test was conducted, based on the range-finding tests, in 250 ml glass beakers with five concentrations of ivermectin (5.6, 10, 18, 32, and 56 parts per trillion), a water control, and a solvent/water control with four replicates of each. Five organisms were placed in each of the 20 test solutions, four water controls, and four solvent/water controls. The temperature was maintained at 21°C. Mortalities at 24- and 48-hour exposure were recorded. In 48 hours, no

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

mortality was seen at the 5.6 ppt dose, however, 40% mortality was seen at the 10 ppt dose and 65% mortality was observed at the 18 ppt dose.

The 48-hour $LC_{50}$ (with 95% confidence limits) was 0.0158 (0.0127-0.0196) mcg/l (ppb).

For the second laboratory study, a definitive static test of the acute toxicity of ivermectin to the neonate Daphnia magna was performed. Fifteen Daphnia were placed into each 2-liter battery jars containing 0.013, 0.022, 0.036, 0.060, and 0.10 mcg/l (ppb) of ivermectin at 22°C. Water quality criteria were regularly monitored over the 48-hour test.

The 48-hour $LC_{50}$ (with 95% confidence interval) was 0.036 (0.030-0.043) mcg/l (ppb), while the no-discernible-effect concentration through 48 hours was 0.013 mcg/l.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

The no discernible effect concentration is the highest concentration tested at which there were no mortalities or observed behavioral and physical abnormalities (i.e. erratic swimming, flared carapace).

To more nearly reproduce circumstances which exist under actual field conditions, experiments were carried out to determine leachate toxicity to Daphnia following the aqueous leaching of ivermectin and its metabolites from feces of swine dosed with ivermectin. Feces alone and feces mixed 1:1 (w/w) with Iowa silt loam soil and placed on top of a 2 cm depth of this soil were leached for a few days with water. The leachates were assayed for total percolated drug and metabolites, for percent of ivermectin in the leachates, and for toxicity of leachates towards Daphnia magna.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

In the studies carried out with composite swine feces mixed 1:1 with soil (drug-equivalent 278 ppb) the material was placed on top of a soil column 9 cm in diameter and 2 cm deep. Only 4.6% of the drug-equivalent (ivermectin and metabolites) leached in 1150 ml of spring water. The drug-equivalent concentration in the leachate was 1.0 ppb, of which only about 0.7% was ivermectin.

The leachate was divided in half. One portion was aerated for 3 days using compressed air. Aeration did not affect the ivermectin level. The aeration was to allow bacterial degradation of most of the dissolved organics before the Daphnia bioassay. Previous bioassays with leachates through control swine and sheep feces had shown high Daphnia 48-hour mortalities and decreases in the dissolved oxygen levels during the assay. Since there was no ivermectin in the control leachates, the drop

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

in dissolved oxygen was thought to be responsible for the mortalities. Spring water was also used, instead of distilled water as in previous studies, to assure sufficient ionic strength for Daphnia survival. The 48-hour survival in the spring water alone was usually 100%.

The aerated and unaerated solutions were brought up to sufficient volume for assay and were serially diluted for a bioassay using Daphnia magna. A solution of spring water percolated through control swine feces/soil was likewise divided, with one portion aerated. These solutions were serially diluted to serve as controls. The results of the bioassay are presented in Table 4. The 48-hour survival of Daphnia was greater in the aerated composite feces/soil leachate than in the unaerated composite feces/soil leachate. The 48-hour survival rates were a bit lower than expected

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

based upon the level of ivermectin present and the previously determined $LC_{50}$ and no mortality level of 0.02 ppb and 0.01 ppb, respectively. However, the toxicity of the leachate was mainly due to the ivermectin present, with the metabolites being much less toxic than ivermectin.

Swine feces were also leached with spring water. Forty-five grams of composite swine feces (555 ppb total drug-equivalent) was placed into a sintered glass funnel and leached with 360 ml of spring water. A similar portion of control swine feces was placed into a funnel and leached. The total drug-equivalent level in the composite feces leachate was 18.1 ppb, representing 26% of the applied residue. Of this, less than 4% was ivermectin.

The composite and control feces leachates were divided in half. One portion of each was aerated

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

for 3 days to allow bacterial degradation of most of the dissolved organics. Aeration did not affect the ivermectin level in the composite feces leachate. The aerated and unaerated leachates were serially diluted with spring water for a bioassay using Daphnia magna. The control leachates were similarly diluted.

The results of the bioassay are presented in Table 5. Drug related activity in the leachate was at a concentration that required dilution by a factor of 324 to ensure survival of all the Daphnia. Survival in the aerated composite feces solution was slightly better than in the unaerated solution. The 48-hour survival Daphnia correlated well with the ivermectin level and the previously determined 48-hour $LC_{50}$ of 0.02 ppb. As in the case of the swine feces/soil leachates, the 48-hour mortalities were attributed to the ivermectin, whereas the majority of the percolated

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(i) Toxicity to Aquatic Organism (Cont'd)

drug-equivalent, the metabolites, were non-toxic or much less toxic than ivermectin.

Since both the feces column and the feces plus soil column contained 45 g composite swine feces, each column contained 25 µg of total drug-equivalent, of which 9.7 µg was ivermectin. The 360 ml water which percolated through the composite swine feces eluted 26% of the drug-equivalent but only 2.7% (260 ng) of the ivermectin. The 1150 ml water which percolated through the swine feces/soil column eluted only 4.6% of the drug-equivalent and only 0.07% (7 ng) of the ivermectin. Thus, soil removed most of the percolating drug-equivalent, and nearly all of the percolating ivermectin.

Similar results were obtained in previous studies with ivermectin in steer and sheep feces.

Table 4

**Forty-eight Hour Survival Rates of Daphnia magna in Dilutions of Swine Feces/Soil and Control Swine Feces/Soil Leachates**

| Dilution of Leachate[a] | Leachate of MK-0933-Dosed Swine Feces/Soil 12113-66C | | | | | | Leachate of Control Feces/Soil 12113-66D | |
|---|---|---|---|---|---|---|---|---|
| | Unaerated | | | Aerated[b] | | | Unaerated | Aerated[b] |
| | Drug-Equivalent ppb | MK-0933 ppb | 48 Hour Survival, % | Drug-Equivalent ppb | MK-0933 ppb | 48 Hour Survival, % | 48 Hour Survival, % | 48 Hour Survival, % |
| Undiluted | 0.80 | 0.004 | 30 | 0.73 | 0.005 | 60 | 100 | 100 |
| 1/4 | 0.20 | 0.001 | 80 | 0.18 | 0.001 | 85 | 100 | 100 |
| 1/12 | 0.067 | 0.0003 | 100 | 0.061 | 0.0004 | 100 | 100 | 100 |
| 1/36 | 0.022 | 0.0001 | 100 | 0.020 | 0.0001 | 100 | 100 | 100 |
| 1/108 | 0.008 | 0.00004 | 100 | 0.007 | 0.00005 | 100 | 90 | 90 |

[a]Dilutions were made serially.

[b]Aeration was prior to bioassay.

Table 5

**Forty-eight Hour Survival Rates of Daphnia magna in Dilutions of Swine Feces and Control Swine Feces Leachates**

| Dilution of Leachate[a] | Leachate Through the Feces of Swine Dosed with MK-0933 | | | | | | Leachate Through the Feces of Swine Contol, Untreated | |
|---|---|---|---|---|---|---|---|---|
| | 12113-66A | | | | | | 12113-66B | |
| | Unaerated | | | Aerated[b] | | | Unaerated | Aerated[b] |
| | Drug-Equivalent ppb | MK-0933 ppb | 48 Hour Survival, % | Drug-Equivalent ppb | MK-0933 ppb | 48 Hour Survival, % | 48 Hour Survival, % | 48 Hour Survival, % |
| 1/4 | 3.09 | 0.12 | 0 | 3.38 | 0.12 | 0 | 90 | 100 |
| 1/12 | 1.03 | 0.040 | 10 | 1.13 | 0.040 | 80 | 100 | 90 |
| 1/36 | 0.34 | 0.013 | 70 | 0.38 | 0.013 | 100 | 100 | 100 |
| 1/108 | 0.11 | 0.004 | 90 | 0.13 | 0.004 | 100 | 100 | 100 |
| 1/324 | 0.038 | 0.0015 | 100 | 0.042 | 0.0015 | 100 | 100 | 100 |
| 1/972 | 0.013 | 0.0005 | 100 | 0.010 | 0.0005 | 90 | 100 | 80 |

[a]Dilutions were made serially. Protocol called for 1 part leachate and 3 parts spring water (1/4) for the most concentrated sample. Actual dilution was about 1 part in five from 18.1 ppb.

[b]Aeration was prior to bioassay.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects

Data in our files, as well as published data[13], indicate that ivermectin and some analogs are effective against a number of insect pests, phytophagous mites, and soil nematodes.

Several compounds were tested for activity against the Mexican bean beetle (Epilachna varivestis), the Southern armyworm (Spodoptera eridania), the black bean aphid (Apohis fabae), the two-spotted spider mite (Tetranychus urticae), the corn rootworm (Diabrotica undecipunctata) and the rootknot nematode (Meloidogyne incognita). Average percent of kill or feeding inhibition, or an effectiveness rating from 0 (no kill or feeding inhibition) to 10 (complete kill or total inhibition of feeding), were noted for each compound.

Aphid contact and systemic poison tests were made on the black bean aphid while feeding on nasturtium plants grown in 2 1/2 inch pots. Tests were made on aphids that migrated to the test plant within the prior 24 hours. The foliage and aphids were exposed to a spray of the test chemical at 250 ppm while the plant was

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

rotating on a turntable. Immediately thereafter, 21 ml of a 250 ppm stock suspension was poured onto the surface of the soil (25 lb/acre rate).

The plants were held under fluorescent light over a paper collar so dead aphids could be collected. The systemic effects were also tested separately without an accompanying foliar application.

Mite contact and systemic tests were performed on bean plants growing in 2 1/2 inch pots and infected with the two-spotted spider mite 24 hours previously. Plants were dipped in a suspension of the test material at 250 ppm. Immediately afterward, 21 ml of a 250 ppm suspension was poured on the surface of the soil (rate equivalent to 25 lb/acre) to provide both a contact systemic effect. From this test, observations are made on adult kill (initial), immature mite kill (residual) and egg development (failure to hatch). Thesystemic effects were also tested separately without an accompanying foliar application.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

For the Mexican bean beetle, a combination of stomach poison and feeding-deterrent effects was measured on larvae about 5 to 7 days after emerging from eggs. Leaves of young bean plants were removed from the plants by cutting the petioles and were dipped in a suspension of the chemical at 250 ppm in the tests. Petioles of the excised leaves were placed in a water reservoir to maintain leaf turgidity, and 5 larvae were placed upon them as soon as the chemical deposit was dry. Observations were made on the mortality of the beetles and the extent of inhibition of feeding two or three days later. For the Southern armyworm, materials were tested as stomach poisons for 5- to 7-day-old larvae of the armyworm. The larvae were transferred from stock cultures to bean leaves that had been dipped in suspension of the test material. The procedures were essentially as outlined for Mexican bean beetle larvae.

In the corn rootworm test, the formulation was mixed with the soil, and corn seedlings and larvae were introduced 3 days after the soil was treated.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

To test nematicide activity, the rootknot nematode has been chosen as a preferred test subject among some 200 plant parasitic nematodes. This nematode is distributed worldwide on a wide assortment of crops. Although it resides in root tissues as a parasite where it incites formation of galls, it may also survive in the soil for many months as a scavenger.

The test described below was designed to destroy free-living forms and to a lesser extent disinfect gall tissue.

Air-dried soil and sand were blended in a ratio of 2:1, and 7 grams of chopped galls and root tissues from an infected stock of plants was added to each gallon of mixture. The inoculum was blended with the mixture and 130 ml was added to each styrofoam cup (10 oz. size). In the test, 10 ml of a 520 ppm suspension (equivalent to 50 lb/acre) was added to each cup which was then covered with a lid, shaken vigorously 2 hours later to assure uniform distribution, incubated 1 to 2 days and again shaken. The covers were removed and the soil leveled. In the cucumber standard test, four cucumber

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

seeds were sown in each cup and covered with 30 ml of sand to a depth of about 1/4 inch. The sand was then sprinkled with a nutrient solution (Miracle Gro at 1 tsp./gal.) containing a damping-off preventive (Dexon at the rate of 1 tsp. of 35% material/gal.) to permit growth of vigorous, healthy roots. After a holding period of 18-25 days, the roots were washed free of soil-sand and rated according to the severity of infection on a scale of 0 (severe galls) to 10 (no infection). In the tomato translocation test, young plants were transplanted into infested soil and sprayed. After a period of 18-25 days, the roots were scored for galling on a scale of 1 to 10.

The major isomer of ivermectin, 22,23-dihydroavermectin $B_{1a}$ ($H_2B_{1a}$, L-638,709) was tested against the Mexican bean beetle, Southern armyworm, aphids and mites at application levels of 33, 8, 2 and 0.5 ppm. Even at the 0.5 ppm level, $H_2B_{1a}$ produced 100% mortalities against the bean beetle, and adult and immature mites, and 90% mortality after 5 days to the aphids. Against the armyworm, $H_2B_{1a}$ afforded 90% mortality at the 8 ppm level. In the systemic test

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

against aphids and mites, however, $H_2B_{1a}$ produced no mortalities at either 0.38 or 1.5 lb/acre, and only 15% mortalities against the corn rootworm at 3.1 and 12.5 lb/acre. These results indicated little or no uptake of $H_2B_{1a}$ by the plants.

In the standard rootknot nematode test $H_2B_{1a}$ exhibited galling ratings of 9 at or above 0.75 lb/acre, and ratings of 7 to 8 at 0.19 lb/acre (86 g/acre).

As a comparison, avermectin $B_{1a}$ ($B_{1a}$, L-676,895) produced 100% mortalities at 0.5 to 33 ppm application level against the bean beetle, adult and immature mites, and aphids by Day 5. Against the armyworm, $B_{1a}$ afforded 100% mortality at the 8 ppm level, but only 40% mortality at 2 ppm. Subsequent tests indicated $B_{1a}$ was active against the bean beetle at an application level of 0.2, but began to lose its activity below that level. Also, subsequent trials showed $B_{1a}$ to cause only about 50% mortalities at the 0.5 ppm level against aphids on Day 5. Also, the

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

activity against the spider mite fell off below 0.2 to 0.5 ppm. $B_{1a}$, like $H_2B_{1a}$, was also ineffective in the systemic test against aphids and mites at 0.38 and 1.5 lb/acre and against the corn rootworm at 3.1 and 12.5 lb/acre, again indicating little or no uptake of the compound by the plants.

In the standard rootknot nematode test with cucumber seedlings, $B_{1a}$ had similar activity to that of $H_2B_{1a}$, demonstrating galling ratings of mostly 9 to 10 at levels down to about 0.75 lb/acre and galling ratings of 8 to 9 at 0.19 lb/acre. In the tomato translocation test, the galling ratings were 10 (no infection) at 0.75, 1.5 and 3.1 lb/acre.

Another compound, related to $H_2b_{1a}$ via the loss of one oleandrose unit, $H_2B_{1a}$-monosaccharide ($H_2B_{1a}$-MS, L-638,724) was also active against the bean beetle, the aphid and mites in the application range of 0.2 to 33 ppm. This compound displayed better activity than $H_2B_{1a}$ towards the Southern armyworm, displaying 100% mortality at 0.2 ppm. The $H_2B_{1a}$-MS

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

also displayed greater than 90% mortalities against adult two-spotted spider mites down to 0.05 ppm, although it was not effective against immature mites at this level. The $H_2B_{1a}$-MS, like $H_2B_{1a}$ and $B_{1a}$, was not effective against aphids or mites when applied systemically at 0.38 or 1.5 lb/acre, or against corn rootworm in the soil even at 3.1 or 12.5 lb/acre.

In the standard rootknot nematode test with cucumber seedlings, $H_2B_{1a}$-MS displayed galling ratings of 9 at 3.1 and 0.75 lb/acre.

A fourth compound, related to $H_2B_{1a}$ by the loss of two oleandrose units $H_2B_{1a}$-aglycone ($H_2B_{1a}$-AG, L638,723) was tested at an application rate of 0.5 ppm against the Mexican bean beetle, where it displayed a mortality rating of zero. Against the Southern armyworm, applications of 0.5 and 0.25 ppm showed conflicting results in two tests, having no mortality at 0.5 ppm, but a mortality rating of 9 at 0.25 ppm. At 0.1 ppm, there was no activity against aphids or mites, a level where $H_2B_{1a}$-MS and $B_{1a}$ were

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(j) Toxicity to Nematodes, Arachnids and Insects (Cont'd)

active. Against the corn rootworm, a level of 0.19 lb/acre in the soil was inactive. In two trials of the standard rootknot nematode test with cucumber seedlings at 0.19 lb/acre, $H_2B_{1a}$-AG displayed galling ratings of only 3 to 5.5.

Thus, toward a variety of insect pests and nematodes, the activities of $H_2B_{1a}$, $B_{1a}$ and $H_2B_{1a}$-MS were quite similar. Less data was accumulated on $H_2B_{1a}$-AG, but it appeared less active than the other compounds against the Mexican bean beetle, the Southern armyworm, aphids and mites. Both $B_{1a}$ and $H_2B_{1a}$-MS were more active than $H_2B_{1a}$ against the Southern armyworm. None of the compounds was active against the corn rootworm, the black bean aphid or the two-spotted spider mite when applied to or mixed into the soil, indicating that the compounds were not readily taken up by the plants. All the compounds showed high activity against Mexican bean beetle larvae and southern armyworms. Exceptionally high activity was observed against aphids and mites.

Table 6: TOXICITY TO NEMATODES AND INSECTS

| Compound | APPLICATIONS TO PLANTS, ppm | | | | APPLICATIONS TO SOIL, lbs/acre | | | |
|---|---|---|---|---|---|---|---|---|
| | Mexican Bean Beatle | Southern Army Worm | Aphids | Two-Spotted Spider Mite | Aphids | Two-Spotted Spider Mite | Corn Root Worm | Root Knot Nematode[c] |
| 22,23-dihydroavermectin $B_{1a}$ ($H_2B_{1a}$, L-638,709) | < .5 | 8 | 0.5 | < .5 | >1.5 | >> 1.5[a] | >12.5 | 0.75 |
| Avermectin $B_{1a}$ ($B_{1a}$, L-676,895) | 0.2 | 2-8 | 0.5 | 0.005 | >1.5 | >> 1.5[a] | >12.5 | 0.2-0.75 |
| 22,23-dihydroavermectin $B_{1a}$ Monosaccharide ($H_2B_{1a}$-MS L-638,724) | 0.5 | 0.005-0.2 | 0.2-0.5 | 0.05 | 1.5 | >> 1.5[a] | >12.5 | .75 |
| 22,23-dihydroavermectin $B_{1a}$ Aglycone ($H_2B_{1a}$-AG L-638,723) | >> 0.5[a] | > .5[b] | >>0.1[a] | >> 0.1[a] | N.T.[d] | N.T.[d] | >> .2 | > .5 |

a Zero mortalities at this level, higher levels not tested.
b No mortality at this level, some mortalities at lower level.
c Level for root galling rating of 9 (0 = severe galling, 10 = no galling).
d N.T. = Not tested.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Annelids

Studies were conducted to determine the $LC_{50}$ for ivermectin to the manure worm, Eisenia foetida in artificial soil under controlled laboratory conditions.

In an initial study, ivermectin was used at 0.1, 1.0, 10.0, 100.0, and 1000.0 mg/kg soil in range-finding tests for toxicity to the earthworm. The definitive test was conducted using four replicates at 12, 25, 50, 100, and 200 mg ivermectin/kg soil with four replicate solvent controls. Copper sulfate was used as a reference toxicant. The test soil consisted of 100 g peat, about 50 g bentonite clay, 5 g cow manure, about 10 g $CaCO_3$ (to maintain a pH of 7.0) and quartz sand added to reach a final weight of 1 kg per test replicate.

Ten worms were added to the surface of each test vessel containing 1 kg of the dosed test soil. In one replicate, the worms were weighed individually and in the other three replicates, all 10 worms were weighed as a group. The same replicate was checked for the before and after test weight range. Test vessels were

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Annelids (Cont'd)

covered with watch glasses and maintained at 20°C in continuous light. Mortality was assessed on days 7, 14, and 28. Weights of live worms and moisture content were determined only when the test was terminated (day 28).

The concentration of ivermectin lethal to 50% of the manure worms ($LC_{50}$) was estimated for the 28-day exposure period by the method of Litchfield and Wilcoxson[14] and found to be 315 mg/kg soil. However, the confidence limits could not be determined. No pathological symptoms or behavioral changes in the worms were noted during the definitive test. However, worms in all of the ivermectin-treated soils did not gain as much weight as the control worms, and the worms in the highest dose (200 mg/kg) actually lost weight over the 28-day test period. It is therefore concluded that all of the ivermectin doses tested appeared to suppress rate of weight gain in the test organisms and that this suppression was dose-related.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(k) Toxicity to Annelids (Cont'd)

An approximate one hundred thousand-fold difference exists between $LC_{50}$ level for manure worms and the environmental burden expected to exist in the soil as a result of fertilization with manure at 20 tons/acre from swine treated with ivermectin at the intended use level.

(l) Bioconcentration by Bluegill Sunfish

A dynamic 42-day study was conducted to evaluate the bioconcentration of $^{3}H$-Avermectin $B_{1a}$ by bluegill sunfish (Lepomis macrochirus). A flow-through proportional diluter system was used to maintain a mean water concentration of 0.099 µg/l $^{3}H$-Avermectin $B_{1a}$ for a 28-day exposure period. Radioanalysis of whole fish, fillet and visceral portions throughout the exposure period indicated a gradual uptake of $^{3}H$-Avermectin $B_{1a}$. Daily bioconcentration factors ranged from 19-69, 6.6-33, and 24-110 for whole fish, fillet, and viscera, respectively. Uptake tissue concentrations of $^{3}H$-Avermectin $B_{1a}$ ranged from 1.9-6.8 ppb for whole fish, 0.66-3.3 ppb for fillet, and 2.4-11 ppb for viscera. The fish ceased accumulating $^{3}H$-Avermectin $B_{1a}$ at about day 10. The compound appeared to have reached a steady-state

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(1) Bioconcentration by Bluegill Sunfish (Cont'd)

plateau as indicated by a linear regression analysis of days 10, 14, 21, and 28 whole fish residue data.

To measure the elimination of $^{3}H$-Avermectin $B_{1a}$, the test fish were placed in clean water for 14 days. Radioanalysis throughout the depuration period indicated 95, 91, and 95 percent clearance rates from whole fish, fillet, and viscera, respectively. The whole fish concentration of $^{3}H$-Avermectin $B_{1a}$ dropped from a day 28 uptake value of 6.8 ppb to 0.32 ppb by day 14 of depuration period. Fillet levels decreased from 3.0 ppb on day 28 to 0.27 by the end of the study; whereas, viscera concentrations dropped from 11 ppb on day 28 to 0.53 ppb by day 14 depuration.

A two-compartment kinetic model was used for analysis of the uptake-depuration whole fish data. The graphical method employed linear regression analysis and yielded an uptake rate constant ($K_1$) of 11 ppb in fish/ppb in water/day, a depuration rate constant

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(l) Bioconcentration by Bluegill Sunfish (Cont'd)

($K_2$) of 0.21 day $^{-1}$, and a calculated steady-state bioconcentration factor (BCF) of 52. This latter value was 75% of the actual day 28 whole fish bioconcentration factor of 69.

(m) Impact of Manufacturing

A secondary environmental effect would result from the discharge of by-products from the chemical manufacturing process for ivermectin.

The following summarizes the environmental effects of manufacture of ivermectin at the Danville, Pennsylvania Plant:

The manufacturing process generates two liquid-waste streams; one a combination of solvent-based waste streams, the other a combination of aqueous waste streams.

The solvent-based waste streams are generated in the isolation step and in the recovery of solvents used for

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

the isolation. They will contain discarded organic by-products and some residual avermectins in a solution of organic solvents such as hexane, ethanol and toluene.

The solvent-based stream will be destroyed by incineration. The incineration process will be subject to and in compliance with the following environmental regulations administered by the Pennsylvania Department of Natural Resources:

Pennsylvania Rules and Regulations for the Protection of Natural Resources, Title 25, Part I, Subpart C, Article I, Land Resources, Chapter 75, Solid Waste Management and Article III, Air Resources.

40 CFR Parts 264 and 265. Standards Applicable to Owners and Operators of Hazardous Waste Treatment, Storage and Disposal Facilities.

The aqueous-based waste stream will consist of the spent fermentation broth and wash waters and will

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

contain unconsumed fermentation nutrients, unrecovered by-products and traces of avermectins and dissolved solvents such as hexane, ethanol and toluene. The aqueous-based stream will be treated in a chemical pretreatment unit designed to destroy residual avermectins; the treated stream will receive final biological treatment in the existing two-stage secondary waste treatment plant and will be discharged under the requirements of and in compliance with NPDES Permit No. PA 0008419 which is administered by the Pennsylvania Department of Natural Resources.

Air emissions generated during the production process will consist of volatile organic compounds such as hexane, ethanol and toluene which will be controlled as appropriate by condensers. The air emissions will be subject to and in compliance with the regulations for air emissions of the Pennsylvania Department of Natural Resources (Title 25, Part I, Subpart C, Article III, Air Resources).

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

Dry solid wastes generated during the production operations (paper, trash, etc.) will be disposed of in an incinerator which will be subject to and in compliance with the regulations for air emissions of the Pennsylvania Department of Natural Resources (Title 25, Part I, Subpart C, Article III, Air Resources).

Air, liquid and solid waste emissions will comply with the above-mentioned environmental control requirements.

The following summarizes the environmental effects of manufacturing ivermectin at the Barceloneta, Puerto Rico plant.

The manufacturing process generates two liquid-waste streams: one a combination of solvent-based waste streams, the other a combination of aqueous waste streams.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

The solvent-based streams are generated in the chemical processing steps. They will contain discarded organic compounds in a solution of solvents such as ethanol, formamide, toluene and water. The solvent-based stream will be destroyed by incineration. The incineration process will be subject to and in compliance with the following environmental regulations:

Puerto Rico Environmental Quality Board Regulations for the Disposal of Solid Waste and Regulation for the Control of Atmospheric Pollution

U.S. Environmental Protection Agency Regulation, 40 CFR Parts 264 and 265.

The aqueous-based waste stream will consist of wash waters generated by equipment washings. Two holding tanks are provided to contain these washings prior to disposal. Both tanks are installed in a concrete sump. The holding tanks are equipped with sodium

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

hydroxide addition facilities and filters to remove solid ivermectin.

The tanks will be tested daily for ivermectin. The tested contents will normally be pumped out through a filter to the chemical sewer which discharges to the Barceloneta Regional Sewage Treatment Plant (BRSTP). If ivermectin is present in the tanks, the contents will either be chemically pretreated with sodium hydroxide to destroy the ivermectin or be incinerated.

All water discharges from the operating area are directed to the holding tanks to contain any potential spills for treatment.

The holding tanks are installed in a concrete sump. Both tanks are equipped with overflow lines into the sump. In the event of the sprinkler system activation, the tanks will overflow into the sump which has an additional holding capacity of 20,000 gal.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

Discharges to the BRSTP will be under the requirements and in compliance with NPDES Permit No. PR 0021237 which is administered by the U.S. Environmental Protection Agency.

Air emissions generated during the production process will consist of volatile organic compounds such as ethanol, formamide and toluene which will be controlled as appropriate by condensers. Exhaust air in the process building and the formulation and sterile areas will be filtered. Air emissions will be subject to and in compliance with the regulation for air emissions of the Puerto Rico Environmental Quality Board Regulations for the Control of Air Emissions.

Dry solid waste, generated during the production operation (paper, trash, etc.) will be disposed of in an incinerator which will be subject to and in

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

compliance with the regulations for air emissions and solid waste disposal of the Puerto Rico Environmental Quality Board.

Ponders End, England Plant

The manufacturing process is guarded against contamination of the environment with respect to gaseous, liquid and solid materials, in the following way:

The solvents (toluene, ethanol and formamide) are handled in atmospheric tanks vented via flame arresters to a safe location. Hazards inherent in the use of hydrogen are minimized by maintenance of a low inventory and use in an open construction ensuring ready disposal should a leak occur.

Where the avermectin intermediate (C-076) is handled, a ventilated glove box is used which has its exhaust connected to a water scrubber. Water from the scrubber

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

is drained to the captive drain system. The product handling area is maintained under negative pressure by an extraction system fitted with a special filter arrangement to prevent product dust release to atmosphere.

In-process materials and solvents are handled in vessels fitted with water-cooled condensers operating on reflux.

Aqueous effluents from the plant and from captive drains are pumped to a holding tank where the pH is adjusted to greater than 12 with caustic soda, the liquors heated to 85 and recycled for 2 hours prior to analysis for residual ivermectin.

Liquors are discharged to the site effluent system at less than 2 ppm of ivermectin.

This is determined by techniques sensitive down to 1 ppm.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

Average total effluent from the site is approximately 100 times greater than the discharge rate from this plant and all effluent passes through equalizing basins before discharge, thus ensuring a high degree of dilution of the already low concentration of ivermectin.

Organic wash liquor residues are disposed of by incineration. Solid waste in the form of contaminated clothing and equipment is sealed in polyethylene plastic bags and similarly treated under direct supervision. Catalyst material recovered as a thiourea complex is collected in a bag filter, solvent washed, and then sealed in a polyethylene container for eventual transfer to the original supplier for metal recovery and refining, the first stage of which is controlled incineration.

The major pieces of legislation controlling environmental emissions in the United Kingdom are:

A) Control of Pollution Act 1974: This mainly deals with disposal of waste, pollution of water, pollution of the atmosphere and noise.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

B) Health & Safety at Work Act 1974: This mainly deals with maintaining a safe working environment but also the prevention of emission into the atmosphere of noxious or offensive substances.

C) Alkali etc., Works Regulation Act 1906 with orders of 1966 and 1971: This is a more specific Act designed to regulate production of certain substances but also to control the emission of specific fumes and gasses.

D) Clean Air Act & Public Health Act could also apply but are of more general application and more often than not utilized by local authorities to prevent nuisance from boiler smoke, etc.

1) Disposal of Solid or Sludge Wastes

This is normally entrusted to a licensed contractor who will analyze such waste and devise suitable means of disposal within the authority granted to him by the local and national inspectorates in the area of disposal. Means of destruction could be: land fill, disposal at sea, incineration with or without exhaust stack scrubbing. In the case of ivermectin all solid waste, including discarded

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

protective clothing, cleaning cloths, etc., are collected in plastic sacks, sealed and periodically incinerated at a municipal incinerator under our direct supervision. The incinerator administration have been fully informed of the nature of the material handled and have made no special requirements with respect to this waste.

2) Disposal of Liquid Effluent

All our liquid effluent streams from the ivermectin plant are neutralized where necessary with lime and discharged via settling tanks to the municipal sewer. Settled sludges are disposed of as in 1. above. Discharge to sewer is governed by consent agreements with the sewage treatment authority who regularly sample and analyze our effluent. In the event of violation of the agreed limits, the authority is empowered to revoke consent agreements or take other action against the offenders. We make no effluent discharge to canals or rivers. All liquid effluent streams from the ivermectin plant are collected separately from all other site waste streams. Aqueous waste including floor washings and

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

roof rain water collections as well as process streams, are filtered and then treated with sodium hydroxide to pH 12 at 80° until samples show an acceptable level of ivermectin has been achieved. At this point the effluent is discharged to the normal site effluent treatment plant where it is diluted with the much greater stream from the rest of the site.

Non-aqueous liquid waste consisting mainly of organic solvents is collected separately and periodically disposed of via a licensed contractor who will incinerate on duly approved premises.

3) Discharge to the Atmosphere

Local authorities are empowered to require the provision of estimates of emission of pollutants or other substances into the air but the major legislation requirement is embodied within the Alkali Act. The premises are subject to periodic visits by the Alkali Inspectorate with specific reference to the emission of HCl, $HNO_3$, $SO_2$,

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

$NO_2$, acetic acid, acetic anhydride and chlorine and its compounds.

No specific vapors are released from this plant to atmosphere which would come under the provision of the Alkali Act. All plant ventilation equipment discharges to atmosphere through hepa filter screens which are periodically replaced. The discarded screens are being incinerated with other solid waste.

4) Noise

Under the Control of Pollution Act, the local authority is empowered to inspect, and where satisfied that a nuisance exists, take such action as may be appropriate to require that noise abatement measures are taken.

Additionally, under the Health & Safety at Work Act, government inspectors may require compliance with a published code of practice or, in the future, regulations which have not yet been published but which are now in the consultative stage.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

Haarlem, Holland Plant

The following procedures are employed to monitor and control environmental emissions and occupational exposure to ivermectin.

1) Weekly monitoring of dust level in presolution room where ivermectin powder is handled.

2) Pending results of activated charcoal treatment, all waste water is temporarily being incinerated.

3) Blood tests of all employees working in ivermectin production every three months and later on every six months.

4) Swab tests every two weeks on equipment, floors and production bottles in production area.

5) Swab test every month from hands of Packaging personnel.

MERCK SHARP & DOHME B.V. at Haarlem, the Netherlands operates regarding environmental matters within the Environmental Pollution Act.

1) Liquids from the ivermectin manufacturing processes are all collected and treated with a charcoal purification unit before entering the plant's general waste system, which also includes domestic sewage waste.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

This goes via a neutralization tank (pH 6-8) and via the municipal sewage system to the Municipal Sewage Water Treatment Plant.

This plant operates under the control of the Hoogheemraadschap Rijnland. M.S.D. has a permit from the municipality for entering the sewage treatment plant with their plant-effluent.

2) Air emissions from the process fall under the State Rules and Regulations Act with regard to Environmental Pollution. The regulations are administered by the Haarlem Department of Environmental Control.

3) Charcoal from the filter system within the charcoal treatment system is collected in plastic bags, put into drums and shipped for incineration. All other collected waste from this factory is combined with

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(m) Impact of Manufacturing (Cont'd)

plant trash and transferred by closed vehicle to the Rijnmond or Alkmaar incinerator. A yearly permit for transport and incineration, issued by the Provincial Environmental Control Agency, under the Law regulates transport and processing solid wastes.

4) With regard to noise, regulations require a working climate, in which 85 dB is maximal. In case the noise level exceeds 85 dB, protective measures have to be provided to all personnel.

3. Describe the Probable Adverse Environmental Effects that Cannot Be Avoided

Based on the discussion in Section D 2, it is not anticipated that any substantial adverse effect on the environment will occur when the new animal drug application for ivermectin is approved. Of course, any manufacturing process must make some contribution of products to the environment. However, as indicated in Section D 2, the liquid, solid and air disposal of by-products from the manufacturing process is done under the applicable environmental requirements of various laws. Furthermore, such wastes from the ivermectin process would make a negligible contribution to the waste problem of modern industrial society.

Prior to the development of effective therapeutic agents, the control of parasitic infections was limited to management systems of pasture rotation and the use of harsh and often injurious chemicals.

Ivermectin is a substantial advance over currently-used products to control swine parasites. High efficacy has been consistently demonstrated against Psoroptes, the causative agent of scabies in swine.

3. Describe the Probable Adverse Environmental Effects that Cannot Be Avoided (Cont'd)

Furthermore, Haematopinus, the swine louse, has been shown to be safely controlled at any stage of its life cycle. Clearly, the simultaneous control of both internal and external parasites with subsequent savings in time and labor reflect an unprecedented advance in swine parasite control.

Ivermectin's potent broad-spectrum, parasiticidal activity establishes this animal drug as having the advantage of controlling a large number of species of parasites. The quantities of undesirable compounds reaching the environment following production and utilization of this new animal drug will be small and environmental effects are not expected to occur.

4. Evaluate Alternatives to the Proposed Action

There is no practical alternative to the use of chemotheraputic agents in controlling parasitism.

4. Evaluate Alternatives to the Proposed Action (Cont'd)

Prior to the development of effective therapeutic agents, the control of parasitic infections was limited to management systems of pasture rotation and the use of harsh and often injurious chemicals.

Ivermectin is a substantial advance over currently-used products to control swine parasites. High efficiency has been consistently demonstrated against lice mites, lungworms and gastrointestinal roundworms of swine. The simultaneous control of both internal and external parasites with subsequent savings in time and labor reflect an unprecedented advance in swine parasite control.

Ivermectin's potent broad spectrum, parasiticidal activity establishes this animal drug as having the advantage of controlling a large number of species of parasites while exposing the environment to a minimal amount of compound.

5. Describe the relationship between local short-term uses of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity

Short-term effects upon the environment, as discussed in Section D 2 (phytotoxicity to a variety of plants, hazard to fish, earthworms, aquatic organisms, etc.), are not expected due to the low levels of noxious compounds which will be present in the environment. Also, as discussed, there would be minimal short-term effect of the disposal of by-products from the manufacturing process upon the productivity of the environment.

These same factors also would mitigate against any long-term detrimental effects on the environment.

Short- and long-term beneficial effects from the use of ivermectin could be substantial in terms of producing healthier swine, allowing swine to realize their full genetic potential to utilize feed more efficiently, eliminate losses from morbidity and mortality from parasite infection. Taken together, this means that more food for man (pork protein) can be produced per pound of feed without increasing the need for such feed and the resulting expenditure of energy.

6. Describe any irreversible and irretrievable commitment of resources

The raw materials used to manufacture ivermectin are common organic compounds -- all of which are in ample supply. Energy commitment would be nominal. Also, some of the raw materials used in the process are recycled or recovered for re-use. Though some of the raw materials are irretrievable, the proportion used in the ivermectin process compared to the total annual production of them would be minimal.

7. Discuss the objections raised by other agencies, organizations or individuals

We know of no agencies, organizations or individuals who have questioned the effect on the environment from the use of ivermectin to treat and control internal and external parasites in swine.

8. If the proposed action should be taken prior to 90 days from the circulation of a draft environmental impact statement or 30 days from the filing of a final environmental impact statement, explain why

The information presented in this environmental impact analysis report amply documents the position that the approval of the new animal drug application for ivermectin by the Food and Drug Administration does not constitute a major agency action which would significantly affect the quality of the human environment. Thus, there is no reason for the Agency to prepare and circulate for comments a Draft Environmental Impact Statement.

9. Analyze whether the benefit to the public of the proposed action will outweigh the action's potential risk to the environment

The benefits to be obtained from the use of ivermectin as discussed in Sections 2 and 5 outweigh any potential risk to the environment.

The risk to the environment can scarcely be identified whereas the benefit in terms of savings from economic loss to the swine producer and the consumer are substantial.

E. Certification

The undersigned applicant/petitioner certifies the information furnished in this Environmental Impact Analysis Report is true, accurate and complete to the best of his knowledge.

Date: JUN 3 0 1985

Kenneth D. Davis
(Signature)

Director, Regulatory Affairs
(Title)

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